Vol. 38, No. 4 December, 1951

THE JOURNAL OF BIOCHEMISTRY

EDITED FOR THE JAPANESE BIOCHEMICAL SOCIETY

PUBLISHED QUARTERLY

BY

THE JAPANESE BIOCHEMICAL SOCIETY

Tokyo University, Tokyo

PRINTED IN JAPAN

The Journal of Biochemistry, Vol. 38, No. 4.

ON THE SPECIFICITY OF ANEURINASE. II.*

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(Received for publication, July 7, 1951)

The mechanism of thiamine destruction by shell-fish aneurinase has been demonstrated in paper I (*1*). It was clarified that thiamine molecule was split into pyrimidine and thiazole nuclei by the attack of shell-fish aneurinase (*2*, *3*, *4*,) and that the amino group of pyrimidine part of thiamine was essential in the process of this enzymatic decomposition.

Here a report will be made on the mechanism of thiamine destruction by bacterial aneurinase and the results of experiments to determine whether and what kind of substrate among 10 thiamine derivatives is decomposed.

Experiments carried out by thiochrome and diazo methods as described before (*4*), has shown that (a) thiamine is split into pyrimidine and thiazole by bacterial aneurinase (see Table I); (b) the essential groups in thiamine to be decomposed into two groups by bacterial aneurinase are free amino group of pyrimidine nucleus of thiamine and quarterly nitrogen in thiamine, while other groups in pyrimidine or thiazole nucleus have little influence on hydrolytic decomposition just as in the case of shell-fish aneurinase as shown in Tables II and III in the experimental part.

In these respects the mode of destruction of the oxidative form of thiamine (SSB_1)** (*5*) by shell-fish aneurinase is different from that by

* Aided by a grant from the Scientific Research Fund of the Department of Education.

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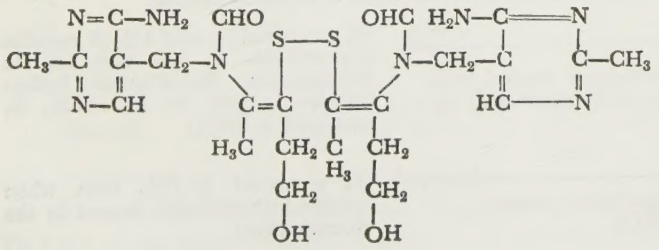

bacterial aneurinase as shown in Table IV. Actually it has been confirmed that SSB_1 can be decomposed easily by shell-fish aneurinase, but hardly by bacterial aneurinase under the same condition.

Moreover, it has been proved that the crude enzyme solution obtained from shell-fish when inactivated by heating, has the property in common with cysteine, by which SSB_1 is changed into thiamine, while bacterial aneurinase has no such action as shown in Fig. 1.

It has been examined whether SSB_1 can be decomposed by shell-fish aneurinase after thiamine formation by the action of cysteine contained in the crude preparation of shell-fish aneurinase. It has been clarified that SSB_1 can be destroyed by shell-fish aneurinase without being converted to thiamine by cysteine, since SSB_1 is destroyed even after cysteine contained in shell-fish has been oxidized by 30% H_2O_2, as shown in Fig. 2. On the other hand bacterial aneurinase is found to be unable to decompose SSB_1, if it is not previously converted to thiamine by cysteine hydrochloride.

FIG. 1.

Results of the experiment to prove cysteine action in crude shell-fish aneurinase and crude bacterial aneurinase

SSB_1 ——————————→ B_1 (B_1 recovered is readily 100%, even when cysteine hydrochloride is not used in the determination)
Shell-fish aneurinase inactivated by heating.

SSB_1 ——————————→ B_1 (B_1 recovered is 0%, if cysteine hydrochloride is not used in the determination)
Bacterial aneurinase inactivated by heating.

FIG. 2.

Results of the experiment to prove the action of shell-fish aneurinase and bacterial aneurinase upon SSB_1 in the absence of added cysteine

SSB_1 ——————————→ B_1 (B_1 recovered is only 4%, if cysteine hydrochloride is not used in the determination. But if cysteine hydrochloride is used to test SSB_1, B_1 recovered is 101%)
Shell-fish aneurinase treated with 30% H_2O_2 and inactivated by heating.

SSB_1 ——————————→ destroyed (B_1 recovered is 0%, even when cysteine hydrochloride is used in the determination)
Shell-fish aneurinase treated with 30% H_2O_2.

EXPERIMENTAL

Preparation of the Crude Bacterial Aneurinase and Crude Shell-Fish Aneurinase.—Bacillus thiaminolyticus was cultivated at 37° for 72 hrs. in sterilized broth. The components of the broth were as follows: meat extract (500 g. of meat were warmed with 400 ml. of water at 50° for 30 min., and then filtered. The filtrate was used as meat extract); pepton, 10 g.; NaCl, 5 g.; filled up to 1000 ml. with water. After the cultivation, the film of the bacteria was separated from the broth by centrifugation. The clear supernatant liquid thus obtained was used as the crude bacterial aneurinase. The crude aneurinase from shell-fish was prepared as in the previous report (*1*). Enzymatic activity of these liquids was tested, using thiamine as substrate for each solution.

Test for Thiamine Destruction by the Crude Bacterial Aneurinase.—To 1～3 ml. of broth containing bacterial aneurinase, 1.9 ml. of phosphate buffer at pH 6.4, and 30γ/3 ml.～60γ/6 ml. of thiamine aqueous solution were added. After the incubation for 2 hours at 37°, each reaction mixture was filtrated. The content of thiamine remained in the filtrate was then determined by both diazo and thiochrome methods. The experimental results are shown in Table I.

TABLE I

Decomposition Test by Thiochrome and Diazo Method on Thiamine Incubated with Bacterial Aneurinase

Expt. No.	Thiamine added γ	Thiochrome method		Diazo method	
		Thiamine remained γ	Decomposition %	Thiamine remained γ	Decomposition %
1	30	2.41	91.1	—	—
2	"	1.21	96.0	—	—
3	"	0	100	0	100
4	60	0	100	0	100
5	50	0	100	0	100
6	"	0	100	0	100
Remark		0.3γ of B_1 in 0.1 ml. of reaction mixture was determined		10～18γ of B_1 in 3 ml. of the reaction mixture were determined	

Decomposition Test for Thiamine Analogues by Bacterial Aneurinase.—To 1 ml. of the crude enzyme solution, 4～8 ml. of phsohate buffer at pH

6.4, and 50γ/ml.~100γ/ml. of substrate (thiamine or its analogues) were added. For blank test the enzyme solution previously heated at 80° for 20 min. was used. After the incubation for 2 hours at 33~37°, the reaction mixtures were heated at 80° for 10 min. and adjusted to pH 4.5. The content of thiamine or its analogues remained in the reaction mixture was determined by the method of diazo-reaction or thiochrome-reaction as described in paper I (*1*). The amount of thiamine and its analogues decomposed by the aneurinase was then calculated by subtracting the amount of substrate remaining in the broth from that added originally. The results of experiments are shown in Tables II and III.

TABLE II

Result of Decomposition Test on Thiamine Analogues Incubated with Bacterial Aneurinase

No.	Substrate	Decomposition by enzyme
I	4-methyl-5-β-hydroxyethyl-N-[(2′-methyl-4′-hydroxy-pyrimidyl-5′)-methyl]-thiazolium-chloride-hydrochloride	−
II	4-methyl-5-β-hydroxyethyl-N-[(2′-methyl-4′-amino-methyl-pyrimidyl-5′)-methyl]-thiazolium-chloride-hydrochloride	−
III	4-methyl-5-β-hydroxyethyl-N-[(2′-hydroxy-methyl-4′-amino-pyrimidyl-5)-methyl]-thiazolium-chloride-hydrochloride	+
IV	4-methyl-5-β-hydroxyethyl-N-[(2′-ethyl-4′-amino-pyrimidyl-5′-)-methyl]-thiazolium-chloride-hydrochloride	+
V	4-methyl-5-β-hydroxyethyl-N-[(2′-methyl-4′-amino-pyrimidyl-5′)-methyl]-thiazolium-chloride-hydrochloride-acetate	+
VI	4-methyl-5-carboethoxy-N-[(2′-methyl-4′-amino-pyrimidyl-5′)-methyl]-thiazolium-chloride-hydrochloride	+
VII	4-methyl-5-chlorethyl-N-[(2′-methyl-4′-amino-pyrimidyl-5′)-methyl]-thiazolium-chloride-hydrochloride	+
VIII	4-methyl-5-β-hydroxyethyl-N-[(2′-methyl-4-amino-pyrimidyl-5′-)-methyl]-thio-thiazolon-(2)-hydrochloride	−

TABLE III

Decomposition Test on Thiamine Analogues Incubated with Bacterial Aneurinase

Expt. No.	Test	Substrate	Content of substrate in the reaction mixture γ/ml.	Diazo method: Broth containing aneurinase ml.	Diazo method: Substrate (calculated thiamine) γ	Diazo method: Substrate recovered γ	Diazo method: Recovery %	Diazo method: Decomposition %	Thiochrome method: Broth containing aneurinase ml.	Thiochrome method: Substrate (calculated thiamine) γ	Thiochrome method: Substrate recovered γ	Thiochrome method: Recovery %	Thiochrome method: Decomposition %
1	Test	I	10	4	40	37.6	—	2.6					
	Blank		"	"	"	38.6	96.5	—					
2	Test		"	"	"	37.0	—	0.6					
	Blank		"	"	"	37.2	93.0	—					
4	Test		5	"	20	18.3	—	10.3					
	Blank		"	"	"	20.4	102.0	—					
5	Test		"	3	15	16.6	—	0					
	Blank		"	"	"	16.6	110.6	—					
									Thiochrome reaction negative				
5	Test	II	"	"	"	16.6	—	0					
	Blank		"	"	"	16.6	110.6	—					
4	Test		"	4	20	19.9	—	0					
	Blank		"	"	"	19.9	99.8	—					
									Thiochrome reaction negative				
3	Test	III	—	—	—	—	—	—	0.1	0.5	0	—	100
	Blank		—	—	—	—	—	—	"	"	0.5	100	—
6	Test		5	3	15	12.0	—	80.0	—	—	—	—	—
	Blank		"	"	"	15.0	100	—	—	—	—	—	—
4	Test		"	4	20	7.8	—	61.0	—	—	—	—	—
	Blank		"	"	"	20.0	100	—	—	—	—	—	—
7	Test	IV	"	—	—	—	—	—	0.1	0.5	0	—	100
	Blank		"	—	—	—	—	—	"	"	0.51	102	—
8	Test		"	—	—	—	—	—	"	"	0	—	100
	Blank		"	—	—	—	—	—	"	"	0.5	100	—
10	Test		"	4	20	0	—	100	—	—	—	—	—
	Blank		"	"	"	19.2	96.0	—	—	—	—	—	—
3	Test	V	"	—	—	—	—	—	0.1	0.5	0	—	100
	Blank		"	—	—	—	—	—	"	"	0.5	100	—
10	Test		"	4	20	0	—	100	—	—	—	—	—
	Blank		"	"	"	22.4	112.0	—	—	—	—	—	—
4	Test	VI	"						0.2	1.0	0	—	100
	Blank		"	Diazo reaction negative					"	"	1.0	100	—
4	Test		"						"	"	0	—	100
	Blank		"						"	"	1.0	100	—
5	Test	VII	"						0.1	0.5	0	—	100
	Blank		"	Diazo reaction negative					"	"	0.5	100	—
4	Test		"						0.2	1.0	0	—	100
	Blank		"						"	0	1.0	100	—
9	Test	VIII*	"						0.1	0.5	0.5	—	0
	Blank		"	Diazo reaction negative					"	"	"	100	—
10	Test		"						"	"	0.56	—	0
	Blank		"						"	"	"	112	—

* The determination of the substrate was made by the BrCN thiochrome method (*6*).

Test for SSB_1 Destruction by Aneurinase.—In an attempt to induce the reaction between the active enzyme and SSB_1, 1 ml. of the shell-fish aneurinase or bacterial aneurinase were mixed with 3.5~8.5 ml. of phosphate buffer and 20γ/0.2 ml.~50γ/1 ml. of aqueous solution of SSB_1. For blank test aneurinase was inactivated beforehand as usual. In the case of shell-fish aneurinase the mixtures were incubated at 37° for 2 hours. After the reaction the mixture for main test was heated at 80° for 10 min. and each mixture was adjusted to pH 4.5. The content of SSB_1 remained in the reaction mixture was determined by thiochrome method after being converted into thiamine by cysteine hydrochloride as described below. The experimental results are shown in Table IV.

The Method of SSB_1 Determination.—To each of three flasks containing 0.2 ml. of the reaction mixture (containing 0.5γ SSB_1), 150γ/0.5 ml. of aqueous solution of cysteine hydrochloride and 0.5 ml. of citrate buffer at pH 7.4, were added. To one of them, moreover, 0.5γ/0.1 ml. of aqueous solution of SSB_1 was added for the recovery test. They were allowed to stand for 1 hour at 37°, and subsequently subjected to the estimation of thiamine by thiochrome method as usual.

SUMMARY

1. The mechanism of thiamine destruction by bacterial aneurinase and the essential groups in thiamine to be decomposed by aneurinase are found to be the same as those of shell-fish aneurinase. Namely thiamine is split into pyrimidine and thiazole by bacterial aneurinase, and free amino group in pyrimidine ring of thiamine molecule and quartery nitrogen in thiamine are essential for the decomposition of thiamine by bacterial aneurinase.

2. SSB_1 can be easily destroyed by shell-fish aneurinase without being converted to thiamine by cysteine. But SSB_1 is hardly destroyed by bacterial aneurinase unless SSB_1 is converted beforehand to thiamine by cysteine hydrochloride.

3. Crude preparation of shell-fish aneurinase has the same action as that of cysteine, while the broth containing bacterial aneurinase has no such action.

The authoress wishes to express her sincere thanks to Dr. Matsukawa of Takeda Institute, who kindly furnished her with the thiamine analogues used in the experiments, and to Dr. Matsukawa of Niigata University, who kindly furnished her with *Bacillus thiaminolyticus* used in this experiment. Thanks are also du to Prof. Akabori at

TABLE IV

Decomposition Test on SSB_1 Incubated with Shell-Fish Aneurinase and Bacterial Aneurinase

Expt. No.	Kind of aneurinase	Test	SSB_1 content (B_1 is used on the enz. act. test) γ/ml.	Enzyme sol. containing SSB_1 ml.	Quantity of SSB_1 (B_1 quantity on enz. act. test) γ	SSB_1 recovery γ	Recovery %	Decomposition %	Remark
1	Sell-fish aneurinase	(Enz. act.)	3.0	0.2	0.6	0	—	100	
		Test	6.0	0.1	〃	0.15	—	75	
		Blank	6.0	〃	〃	0.60	100	—	
2		(Enz. act.)	5.0	〃	0.5	0.05	—	90	
		Test	〃	〃	〃	0.11	—	78	
		Blank	〃	〃	〃	0.50	100	—	
3		(Enz. ac.t)	2.5	0.2	〃	0	—	100	SSB_1+ $2H_2O$
		Test	〃	〃	〃	0.05	—	90	
		Blank	〃	〃	〃	0.50	100	—	
4		(Enz. act.)	〃	〃	〃	0	—	100	
		Test	〃	〃	〃	0	—	100	
		Blank	〃	〃	〃	0.46	92	—	
5		(Enz. act.)	〃	0.4	0.1	0	—	100	
		Test	〃	0.2	0.5	0	—	100	
		Blank	〃	〃	〃	0.47	94	—	
6	Bacterial aneurinase	(Enz. act.)	5.0	0.1	〃	0	—	100	
		Test	〃	〃	〃	0.5	—	0	
		Blank	〃	〃	〃	0.5	100	—	
7		(Enz. act.)	〃	1.0	〃	0	—	100	
		Test	〃	〃	〃	0.5	—	0	
		Blank	〃	〃	〃	0.5	100	—	
8		(Enz. act.)	〃	0.1	〃	0	—	100	
		Test	〃	〃	〃	0.5	—	0	
		Blank	〃	〃	〃	0.5	100	—	
9		(Enz. act.)	〃	〃	〃	0	—	100	SSB_1+ $2H_2O$
		Test	〃	〃	〃	0.5	—	0	
		Blank	〃	〃	〃	0.5	100	—	
10		(Enz. act.)	〃	〃	〃	0	—	100	SSB_1+ $2H_2O$
		Test	〃	〃	〃	0.44	—	4.4	
		Blank	〃	〃	〃	0.44	92	—	
11		(Enz. act.)	〃	〃	〃	0	—	100	SSB_1+ $2H_2O$
		Test	〃	〃	〃	0.5	—	0	
		Blank	〃	〃	〃	0.5	100	—	

Osaka University for his painstaking revision of the paper and to Dr. Chachin, Director of this Research Institute for his support in executing this work.

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The Journal of Biochemistry, Vol. 38, No. 4.

ON THE PATHOGENESIS OF PORPHYRIA, ESPECIALLY OF THE TOXIC PORPHYRIA AFTER THE ADMINISTRATION OF SULFONAL DERIVATIVES

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(Received for publication, July 11, 1951)

Porphyrin has hitherto been considered as a harmful substance, that needs to be detoxicated in body or excreted from body. Consequently, the porphyrinuria has been regarded as the result of the increases of porphyrin in body due to either its excess formation or its insufficient elimination, and the symptoms of the porphyria, the extreme case of such porphyrinuria, have been all attributed to the toxic action of porphyrin.

According to the results of the author's experiment (*1*, *2*), porphyrin is however, not only a harmless substance but also a very important constituent of all cells, either free or combined with metals, the amount of which contained in the cells is an indicator of the functional state of cell and is controlled at every moment by the vegetative nervous system and also by various substances (vitamins, minerals, incretory substances, *etc.*) of the cell surroundings.

Therefore, it is difficult to explain the symptoms of acute porphyria as the toxic action of porphyrin. Moreover, chronic porphyria, in which case the enormous increase of porphyrin in body is ascertained, is lacking in the abdominal symptoms, the changes in the nervous system and the injury of parenchymatous organs. Acute porphyria is seldom, if ever, accompanied by the photosensitization symptoms, which are surely due to the action of the increased porphyrin. Namely, acute and chronic porphyria seem to be of different nature and the pathogenesis of the acute porphyria seems to be not the increase of porphyrin in body. How, then, will be the state?

Such a state as acute porphyria can, by no means, be realized experimentally. As for toxic porphyria, which is often called so because of its similarity to acute porphyria, it can be reproduced in experimental animals. Thereupon, the author has first investigated in the experimental lead porphyria of guinea pigs and has ascertained that lead porphyria

is the extrusion of intracellular porphyrin bodies (porphyrin and metalloporphyrin), especially of metalloporphyrin from cells due to the introduced lead (*1*, *3*).

The author has then experimented on the effect of sulfonal derivatives, its intoxication being accompanied by the toxic porphyria, upon tissue porphyrin bodies of guinea pigs, and obtained interesting results. In the present report the outline of such results will be described and the pathogenesis of acute porphyria will be further discussed.

EXPERIMENTAL

The content of porphyrin bodies in various tissues and organs of guinea pigs was measured by means of the author's method (*1*, *4*, *5*) after the administration of sulfonal derivatives. 0.03 g. per 100 g. body weight of sulfonal mixed with bean curd refuse were given *per os* for 6 days and on the 7th day the content of porphyrin bodies was measured. Also, the measurement was performed at a certain time after the subcutaneous injection of 2 ml. of 1 g./dl. methylsulfonal aqueous solution per 300 g. body weight, namely 0.007 g. per 100 g. body weight, for 1 time or several times (this dose once daily). The sulfonal administration was performed in 1944 and the methylsulfonal administration in 1949.

Table I

Porphyrin Bodies Content of Various Tissues after the Administration of Sulfonal for 6 Days

A guinea pig, 300 g.
Daily 0.09 g. of sulfonal given *per os*.
Porphyrin bodies content in γ/dl. or 100 g. wet weight.

	Liver	Kidneys	Heart	Skeletal muscle	Spleen	Suprarenals	Intestine	Testicles	Skin	Lungs	Cerebrum	Cerebellum	Bone	Bone marrow
Metalloporphyrin	54	51	56	111	381	70	5	5	4	26	5	7	52	
Porphyrin	3	3	3	1	5	8	1	1	1	1	1	3	6	11

Table II

Metalloporphyrin Content of Various Tissues after the Administration of Methylsulfonal

Guinea pigs.

0.007 g. per 100 g. body weight of methylsulfonal given subcutaneously for 1 time (1 time daily).

Porphyrin bodies content in γ/dl. or 100 g. wet weight.

Treatment	Animals			Date	Liver	Kidneys	Heart	Skeletal muscle	Spleen	Suprarenals	Intestine	Testicles	Skin	Lungs	Cerebrum	Cerebellum	Bone	Urine
	No.	Body weight	Sex															
None	Average				**103**	**65**	**178**	**60**	**701**	**365**	**46**	**6**	**8**	**32**	**5**	**4**	**109**	**13**
30 min. after 1 time administration	1	270	♂	20/VII	274	444	348	58	1216	201	25	2	13	1717	0	0	145	7
	2	270	♂	25/VII	302	225	356	60	1257	363	32	2	13	27	1	9	143	2
	3	280	♀	8/VIII	210	423	707	118		407			13	148	1	3	125	8
	Average				**262**	**364**	**470**	**79**	**1237**	**323**	**29**	**2**	**13**	**631**	**1**	**4**	**138**	**6**
120 min. after 1 time administration	4	260	♂	29/VII	**238**	**176**	**408**	**60**	**1435**	**412**	**28**	**2**	**12**	**56**	**1**	**4**	**100**	**20**
48 hrs. after 1 time administration	5	300	♂	10/VIII	**547**	**243**	**717**	**258**	**1949**	**465**	**59**	**397**	**29**	**57**	**1**	**4**	**133**	
24 hrs. after 2 times administration	6	300	♂	18/VIII	**185**	**58**	**191**	**94**	**1225**	**477**	**26**	**178**	**28**	**56**	**1**	**5**	**155**	
48 hrs. after 4 times administration	7	270	♂	1/VIII	26	45	136	60	1520	777	28	7	13	52	1	8	219	21
	8	260	♂	1/VIII	28	47	188	57	1534	457	24	33	12	53	0	4	146	5
	9	300	♀	13/VIII	29	31	145	62	772	442	36		10	57		1	88	42
	Average				**28**	**41**	**156**	**60**	**1275**	**559**	**29**	**20**	**12**	**54**	**1**	**4**	**151**	**23**

TABLE III

Porphyrin Content of Various Tissues after the Administration of Methylsulfonal

Under the same condition as those in Table II.

Treatment	No. of Animals	Liver	Kidneys	Heart	Skeletal muscle	Spleen	Suprarenals	Intestine	Testicles	Skin	Lungs	Cerebrum	Cerebellum	Bone	Bone marrow	Whole blood	Blood plasma	Bile	Urine
None	Av.	**2**	**2**	**4**	**1**	**10**	**21**	**2**	**2**	**2**	**3**	**2**	**7**	**15**	**15**	**51**	**2**	**9**	**8**
30 min. after 1 time adm.	1	3	3	8	3	18	72	3	23	3	3	3	14	55	23	23	5	27	11
	2	3	3	10	2	24	72	3	16	2	3	2	8	24	39	22		31	13
	3	2	2	6	3	19	36	2		2	2	2	10	18	23	23	3	9	12
	Av.	**3**	**3**	**8**	**3**	**21**	**60**	**3**	**20**	**2**	**3**	**2**	**14**	**32**	**28**	**23**	**3**	**22**	**12**
120 min. after 1 time adm.	4	2	2	6	2	20	48	2	17	3	2	2	10	60	36	25	2	28	13
48 hrs. after 1 time adm.	5	**2**	**2**	**5**	**2**	**19**	**39**	**5**	**7**	**2**	**2**	**2**	**10**	**23**	**43**	**24**	**2**	**22**	**14**
24 hrs. after 2 times adm.	6	**2**	**2**	**5**	**2**	**15**	**48**	**2**	**7**	2	**2**	**2**	**8**	**29**	**24**	**23**	**2**	**42**	**43**
48 hrs. after 4 times adm.	7	2	3	7	2	29	44	2	15	2	2	2	9	21	27	36	2	12	24
	8	2	3	3	2	24	38	2	12	2	2	3	12	26	25	36	5	34	24
	9	2	2	6	3	23	39	2		4	2	3	13	33	81	25	2	32	22
	Av.	**2**	**3**	**5**	**2**	**25**	**41**	**2**	**13**	**3**	**2**	**3**	**11**	**27**	**44**	**32**	**3**	**26**	**23**

RESULTS AND DISCUSSION

As shown in Table I (only a representative case is shown), the oral administration of sulfonal for 6 days causes generally very marked decrease of metalloporphyrin content in various tissues other than skeletal muscle and also decrease of the porphyrin content. Porphyrin bodies in body are at all increased not, but rather decreased generally, even to a half in liver. Namely, the sulfonal intoxication can be interpreted as the extrusion of intracellular porphyrin bodies from cells by continuous administrations of sulfonal.

As the cause of the porphyrinuria in case of the sulfonal intoxication the disturbance in the excretion of porphyrin from liver due to liver injury (*6*) or the superfluous formation of porphyrin due to the disturbance in the synthesis of hemoglobin in bone marrow (*7*) has been

hitherto considered. It is quite clear from the above mentioned results that such conceptions are not correct, but the porphyrinuria in this case is just the manifestation of the extrusion of the cellular porphyrin bodies. The liver injury (*8*) in case of the sulfonal intoxication is not the cause of the porphyrinuria, but the rather result of the decrease in porphyrin bodies in liver. Similarly, the renal injury (*9*) can be interpreted as the result of the decrease in porphyrin bodies in kidneys.

To know the course of the development of the sulfonal intoxication, methylsulfonal is parenterally administered, because it is more soluble in water and is said to cause a more marked porphyrinuria (*6*, *10*). As shown in Tables II and III, metalloporphyrin in intestinal tract and porphyrin in whole blood begin to be decreased since the 1st administration, but metalloporphyrin in liver, kidney, heart, *etc.* are increased after the 1st and the 2nd administrations and become to be decreased after the 4th administration. Metalloporphyrin in spleen and suprarenals is increased after the 1st administration and then begins to be decreased, but remains at a value higher than the normal even after the 4th administration.

The changes caused by the sulfonal administration are in such respects quite different from those by lead administration. Sulfonal derivatives can not be said as a factor to cause the decrease in porphyrin bodies directly, rather they must cause the decrease secondarily. At this point the fact that most of the patients of the porphyrinuria caused by the intoxication of hypnotica are female (*9*), is very interesting and leads us to conceive an intimate connection of such a porphyrinuria to the behavior of vegetative nervous system and incretory organs. It should be recalled that the functional state of vegetative nervous system is one of the factors causing the change in tissue porphyrin bodies and also that the parasympathetic excitement decreases tissue porphyrin bodies to cause porphyrinuria (*1*, *5*, *11*). Then, the following interpretation would suggest itself: Sulfonal derivatives would change the functional state of higher centrum (*1*, *11*) at interbrain, which controls the distribution of intracellular porphyrin bodies, and would cause the increase in metalloporphyrin content in various organs, and then later, the decrease when the intoxication begins to develop.

As is clear from the above discussions, two representative cases of the toxic porphyria can be regarded as the result of the escape of intracellular porphyrin bodies, especially of metalloporphyrin from cells. In case of lead porphyria it is due to the factors to promote metalloporphyrin→porphyrin process and also to decrease porphyrin bodies

in cells primarily; and in case of the sulfonal porphyria sulfonal perhaps to the factor to change the functional state of the higher centrum of vegetative nervous system and then to cause the decrease in intracellular porphyrin bodies secondarily.

Accordingly, the pathogenesis of acute porphyria may be conceived similarly as the escape of porphyrin bodies from the cells. The cases reported by Vannotti (*12*) as "Myoporphyrie" in which the decrease in myoglobin is the cause of porphyrinuria are appropriate evidences for such a conception. Liver injury in case of acute porphyria may be well explained as the results of the extrusion of liver porphyrin bodies from liver and can not be considered as the cause of porphyrinuria. Some symptoms of acute porphyria lead us to suppose the existence of disequilibrium of vegetative nervous system in case of acute porphyria. In fact, Iwatsuru (*13*) has reported a case of acute porphyria ascertained to be parasympathetically excited in the pharmacodynamical examination. Also the fits of acute porphyria are often provoked by fever or menses. Further, certain disturbances in the functions of incretory glands and vegetative nervous system can be seen before the fits. It may be, therefore, probable that the disequilibrium of vegetative nervous system is primary.

Thus, the acute porphyria can be interpreted as such a disease in which certain disturbances in the vegetative nervous system causes the marked changes in distribution of porphyrin bodies in the body and as the result of the decrease in tissue porphyrin bodies, porphyrinuria occurs on the one hand and various symptoms appear as porphyrinoprival phenomena on the other.

SUMMARY

1. The oral administration of sulfonal for 6 days causes generally marked decreases in metalloporphyrin content in various tissues other than skeletal muscle.

2. The parenteral administration of methylsulfonal causes the first increases and then later decreases in metalloporphyrin content in various tissues and organs.

3. The pathogenesis of sulfonal porphyria is the escape of intracellular porphyrin bodies, especially of metalloporphyrin from cells.

4. The action of the administered sulfonal on intracellular porphyrin bodies may be the secondary one.

5. The pathogenesis of acute porphyria is further discussed.

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ON THE METABOLISM OF CHOLIC ACID IN ORGANISM

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(Received for publication, July 13, 1951)

F. Mylius (*1*) had found a desoxycholic acid in the putrefactive bile and stated that this acid could be produced from cholic acid by the action of bacteria in putrefactive bile. Exner (*2*) and others had confirmed in the culture medium of *Escherichia coli*, *Eberthella typhosa*, *Klebsiella pneumonia*, that cholic acid had in fact altered so that it did not precipitate by the addition of lead acetate. Bondi (*3*) had observed in the same way that the Pettenkofer reaction of cholic acid in the bile ceased to appear after keeping it in putrefaction for a few days. H. Wieland had expressed a contrary opinion upon the Mylius' experiments, because the separation of cholic acid from desoxycholic acid was very difficult as the latter did not produce any easily separable molecular compounds with another organic substance. Also, Mori (*4*), by adding pure cholic acid in putrefactive pancreas, had found that cholic acid was oxidized and reduced into desoxycholic acid. According to the result of such many experiments (*5*, *6*, *7*) on the bile acid it seems probable that cholic acid could be converted in the animal body through the process of oxidation and reduction into desoxycholic acid or chenodesoxycholic acid and further into lithocholic acid. Recently Morimoto and Shimizu (*8*) has added pure cholic acid to the culture medium of *Bac. coli communis* and found 3,12-dihydroxy-7-ketocholanic acid, but not desoxycholic acid.

The present author, therefore, made some experiments on the change of 3,12-dihydroxy-7-ketocholanic acid in the medium of putrefied pancreas and the results are reported here.

EXPERIMENTAL

Into 2 liters of 1% solution of sodium 3,12-dihydroxy-7-keto-cholanate, well minced fresh pancreas of two hogs was poured and the whole solution was kept at 37° in an incubator for six months and subjected to thorough putrefaction. A dark, brownish solution was obtained. This

was under vacuum concentrated and dried. The dried residue was extracted with alcohol, which was afterward distilled off; the residue was then treated with petroleum-ether in order to extract fat; the remainder was dissolved in water and made alkaline with 5% sodium carbonate solution and filtered. The filtrate was acidified with dilute HCl and the precipitate was extracted with ether. Ether extracts were collected and evaporated, upon which a dark, red, pasty residue was obtained. The residue was treated with petroleum-ether to remove traces of fatty substances and then dissolved in 2% ammonium solution. To this ammonium solution 10% barium chloride solution was added until no more precipitate was produced.

The barium salt was separated into two parts, one soluble in water, and another insoluble. To insoluble barium salt 5% sodium carbonate solution was added and heated on water-bath for 30 minutes, and then filtered. The filtrate was acidified with dilute HCl, upon which a dark, red, brownish precipitate was produced. The precipitate was collected, dried, dissolved in ether and left aside at room temperature. Then a great amount of crystals appeared. These crystals were collected and recrystalised from glacial acetic acid. The yield was 1.7852 g. The crystal had long column-like shape. The melting point was 145°, which was not lowered when mixed with choleinic acid. The results of analysis is as follows.

Analysis: Calcd. for $C_{24}H_{40}O_4$-CH_3COOH; C, 69.25; H, 9.735
Found: C, 69.35; H, 9.813

As to the water-soluble barium salt, its solution was acidified with dilute HCl, upon which precipitate was produced, and this precipitate was dissolved in ether and left aside at the room temperature; a mass of paste-like substance was obtained, but no crystal.

Thereby, following Wieland's method (*9*) this sticky substance was extracted with 15% HCl and the residue was extracted with ether. The ether solution was left at the room temperature, then hard, white crystals were obtained.

This white crystals were further treated with ethyl alcohol and recrystalised. The yield was 1.462 g. It was octahedron crystal, having the melting point 197°, which showed no lowering when mixed with pure cholic acid.

Analysis: Calcd. for $C_{24}H_{40}O_5$: C, 70.53; H, 9.87
Found: C, 70.41; H, 10.08

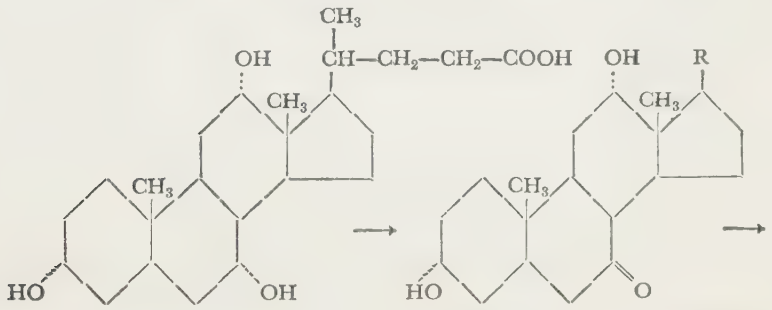
From the filtrate, after the cholic acid had been separated, the solvent.

was distilled off, and the residue was heated with semicarbazide hydrochloride and sodium acetate for five hours according to the established method. By this operation, 0.342 g. of semicarbazone of 3,12-dihydroxy-7-keto-cholanic acid was obtained.

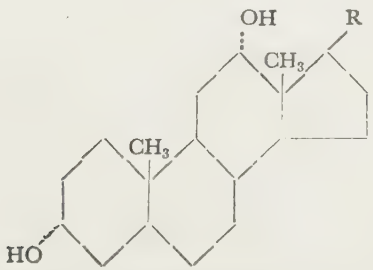
The extract with 25% HCl obtained as above was diluted with water leading to the outcome of the precipitate. This precipitate was extracted with ether, from which a small amount of crystals of choleinic acid was obtained.

SUMMARY

From the culture medium of putrefied pancreas tissue containing 3,12-dihydroxy-7-keto-cholanic acid (II) a great amount of desoxycholic acid (III) and cholic acid (I) were separated. Cholic acid, exactly according to the theory expounded by T. Shimizu, is transformed to desoxycholic acid through the process *via* dehydrocholic acid. But there is also another way *via* 7-keto body as follows.



(I) Cholic Acid (II) 3,12-Dihydroxy-7-keto-cholanic acid



(III) Desoxycholic acid

We have proved the formation of desoxycholic acid from cholic acid in putrefied media and expect that the same process may surely occur in normal animal body. But we must wait for the results of further investigation to determine in what particular organ the oxidation followed by the reduction of cholic acid takes place.

In concluding the experiments the author desires to acknowledge his indebtedness to Prof. Kazuno for this valuable advice and suggestions, and to Prof. Shimizu for his helpful criticism through the course of this work.

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The Journal of Biochemistry, Vol. 38, No. 4.

ENZYMATIC DECOMPOSITION OF NITROMETHANE BY LIVER HOMOGENATES

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(Received for publication, July 23, 1951)

Sato and one of us have shown that nitromethane is decomposed to form nitrite by liver homogenate (*1*). The present paper describes the properties of the enzyme system engaged in this reaction.

MATERIALS AND METHODS

Fresh rabbit liver was homogenized with five volumes of distilled water in a Potter-Elvehjem homogenizer. Because the supernatant liquid of the centrifuged homogenate has little enzymatic activity, the whole homogenate was used without further treatment for the following experiments.

Into Erlenmeyer flasks were pipetted 5.0 ml. of homogenate, 5.0 ml. of 0.2 *M* phosphate buffer (0.2 *M* KH_2PO_4 plus 0.2 *M* Na_2HPO_4), 5.0 ml. of 0.05 *M* nitromethane in aqueous solution and sufficient water to make a total volume of 20 ml. of reactants. 1 ml. of toluene was added to prevent contamination by bacteria. The addition of toluene showed scarcely any influence upon the process (*1*). The reaction mixtures were incubated at 37°.

EXPERIMENTAL RESULTS

Enzymatic Nature of the Reaction—Samples were withdrawn at intervals and deproteinized with cadmium hydroxide (*2*). The nitrite formed was determined colorimetrically with a Pulfrich stufenphotometer using Griess-Ilosvay reagent. The typical results thus obtained are shown (Fig. 1). The large increases in nitrite formation were attributed to the enzymatic action, as a boiled homogenate plus the substrate (control) showed no such stimulation of nitrite formation.

Effect of pH—The optimum pH was determined using 0.2 *M* phosphate buffer in the range pH 5–11. The amount of nitrite formed was

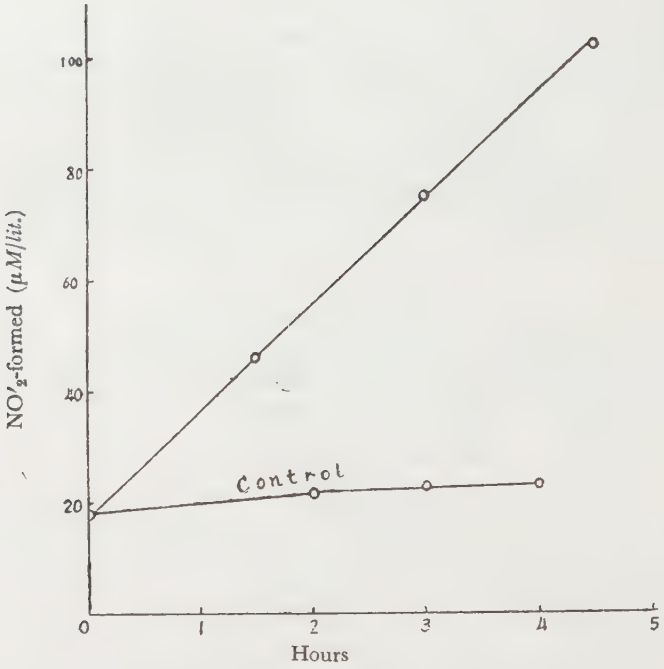

FIG. 1. Nitrite formation by rabbit liver homogenate. Temp., 37°.; pH 8.5.

plotted against the pH of the contents of the individual flask. A rather sharp optimum was found in the region of pH 8.5–9.0 (Fig. 2.).

Effects of Inhibitors—Table I summarizes the results of the action of certain inhibitors on the enzymatic decomposition of nitromethane by rabbit liver homogenate. The figures given represent the percentage activity of the enzyme. The inhibitors were employed in aqueous solution and reaction mixtures have been adjusted to pH 8.5–9.0. Potassium cyanide and sodium azide strongly inhibited the reaction, while urethane did a little. On the other hand, no inhibition and rather an acceleration has been observed with sodium fluoride. As under these conditions hydroxylamine spontaneously produces considerable amount of nitrite, the inhibition by hydroxylamine on the enzymatic nitrite formation was estimated approximately by subtracting the values obtained for control without enzyme.

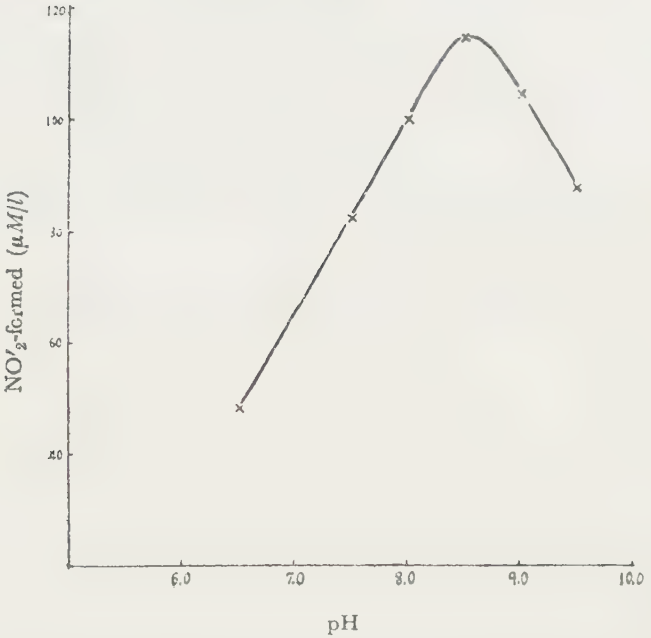

FIG. 2. Effect of pH on the yield of nitrite by liver homogenate. Temp., 37°. Time of incubation, 3 hrs.

TABLE I

Effects of Inhibitors on the Enzymatic Decomposition of Nitromethane by Rabbit Liver Homogenate

Inhibitors	Molar concentration of inhibitors	% Activity
KCN	1×10^{-3}	0
NaN_3	3×10^{-3}	0
NH_2OH	3×10^{-4}	about 80
NaF	3×10^{-3}	110
NaF	3×10^{-2}	140
Ethyl urethane	1×10^{-1}	90

Effect of H_2O_2—Scott (*3*) reported that nitroethane was oxidized by hydrogen peroxide at pH 7, in the presence of ferrous sulfate to form

acetaldehyde and nitrite, and apparently the same oxidation occurred in fresh citrated blood and in the circulating blood of a rabbit after intravenous injection of nitroethane. Therefore, the effect of hydrogen peroxide on the decomposition of nitromethane was examined. In the course of the reaction, 0.5 ml. of hydrogen peroxide in aqueous solution (0.3%) was added every 15 min., employing distilled water as a control. The nitrite formation was not affected at all by the addition of hydrogen peroxide. (Table II).

TABLE II

Effect of Hydrogen Peroxide on the Decomposition of Nitromethane by Liver Homogenate

Temp., 37°; Time of incubation 2 hrs; pH 8.5.

	Nitrite formed
(1) In the presence of H_2O_2	56 *micromoles/lit.*
(2) Control	57

Decomposition Products of Nitromethane—Scott (*3*) showed that both acetaldehyde and nitrite were found in the blood of a rabbit after injection of nitroethane. We have tried to detect decomposition product of nitromethane other than nitrite ion. Formaldehyde, one of the possible products, was not found (Schiff's reagent) in the distillates of the reaction mixture after incubating for 20 hrs. Further, neither methanol nor formic acid was detected.

SUMMARY

Our experiments showed that the nitromethane decomposing enzyme from rabbit liver was strongly inhibited by potassium cyanide and sodium azide. This seems to suggest that the enzyme might be a metalloprotein.

Hydrogen peroxide added in excess to the reaction mixture during incubation exhibited no effect upon the enzyme activity. The result reveals that the nitrite formation in the course of this reaction has nothing to do with the mechanism suggested by Scott nor with the coupled oxidation in the sense of Keilin and Hartree, *et al.* (*4*). Moreover, ethyl urethane known to inhibit dehydrases, by which hydrogen peroxide may be produced, has little inhibition in the reaction. This observation also supports the above consideration.

We have been unable to catch any decomposition products of

nitromethane except nitrite. Having used crude homogenate as a source of the enzyme, it seems that the decomposition product has been subjected to further change.

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STUDIES ON LIPASE. III. ON THE ACTIVATION OF LIVER ESTERASE

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(Received for publication, July 26, 1951)

There are many observations about the distinction of liver esterase from pancreas lipase. It is believed that liver esterase is fitting to the hydrolysis of glycerides of lower fatty acids such as triacetin or tributyrin, but not to those of higher fatty aicds as olive oil.

On the other hand, according to Rona *et al.* (*1*), atoxyl is a specific inhibitor for liver esterase, while quinine a specific inhibitor for pancreas lipase.

Willstätter *et al.* (*2*) found that leucyl-diglycine was able to activate pancreas lipase but not liver esterase.

Furthermore, Glick *et al.* (*3*) have reported that bile acids and oleate, which stimulate pancreas lipase, exert an inhibitory effect on liver esterase.

Referring to the relationship between lipase and the redox-system, Kayashima (*4*) described that the hydrolytic action of liver esterase was accelerated in a reducing medium and retarded in an oxidizing medium, and that the synthetic action was reversely influenced.

In the previous paper the author (*5*) has dealt with the activating effect of amino acids, especially of histidine on the pancreas lipase.

The investigations were then carried out with liver esterase, which was also stimulated by the addition of amino acids, especially of histidine.

It must be here pointed out that the breakdown of olive oil by the liver enzyme is significantly accelerated by the addition of histidine.

EXPERIMENTALS

Influence of Amino Acids on Liver Esterase.—Liver powder as an enzyme source: According to H. Kraut *et al.* (*6*) a fresh pig liver

was ground and dried by the usual acetone-ether method, yielding about 15 g. of the acetone powder from 135 g. of a fresh liver.

The esterase activity was found to exist after more than one year in this liver powder, stored in a desiccator.

A half g. of liver powder was treated with 40 ml. of 70% glycerol-water and kept stirring at 37° for 4 hours.

The extract was centrifuged and the supernatant fluid was used as the esterase source.

Test solutions: 0.17 g. of triacetin or 0.11 g. of butyl-butyrate dissolved in 2 ml. of 0.2 *M* NH_4Cl-NH_4OH buffer (pH 8.6) was added to 2 ml. of esterase solution and 1 ml. of 0.1 *M* amino acid solution, adjusted to pH 8.6.

Control with 1 ml. water without amino acids was examined under the same conditions.

Blanks without substrate or without esterase were similarly carried out.

These solutions were incubated under toluene at 37°. After the incutation of 1 and 24 hours the acidity in 1 ml. of digest was titrated with 0.1 *N* methanolic NaOH solution by using a microburette. The values are corrected for the blanks and the acidity increase is given in ml. of 0.1 *N* NaOH in the tables.

The acidity increase in the test solutions is given as the degree of hydrolysis in per cent for that of the control in the table.

Influence of Derivatives of L*-Aspartic Acid on Liver Esterase* (*Triacetin and Butyl-Butyrate Hydrolysis at pH* 8.6)—The enzyme source and the test procedures were the same as in the previous experiment. 1 ml. of 0.1 *M* L-aspartic acid or its derivative solution was added, resulting a 0.02 *M* final concentration.

Alkali Treatment of Liver Esterase (*Triacetin Hydrolysis at pH* 8.6).—Alkali treatment of the liver powder extract: 2 ml. of glycerol-water extract of the liver powder, prepared as in the preceeded experiment were mixed with 1 ml. of water, adjusted to pH 9.2 or 11.0 with 1 *N* NaOH and incubated at 37° for 24 hours, to be examined for its behavior towards alkali.

In the second case, 2 ml. of glycerol-water extract were mixed with 1 ml. of 0.1 *M* L-aspartic acid solution, adjusted to pH 9.2 with 1 *N* NaOH and previously incubated at 37° for 24 hours, in order to observe the effect of L-aspartic acid on alkali treatment.

In the third case, 2 ml. of glycerol-water extract were rendered

TABLE I

Acidity increase in 1 ml. of digest at pH 8.6 (ml., 0.1 N NaOH)

Substrates / Time (hours) / Amino acids in 0.02 M final concentration	Triacetin				Butyl-butyrate			
	1		24		1		24	
Control (No addition)	0.120	Degree 100	0.610	Degree 100	0.105	Degree 100	0.460	Degree 100
Glycine	0.156	130	0.775	127	0.108	103	0.480	104
L-Leucine	0.160	133	0.825	135	0.110	105	0.475	103
L-Aspartic acid	0.162	135	0.920	151	0.109	104	0.501	109
DL-Aspartic acid	0.162	135	0.920	151	0.109	104	0.501	109
L-Glutamic acid	0.158	132	0.885	145	0.107	102	0.492	107
L-Ornithine	0.163	136	0.903	148	——	——	——	——
L-Histidine	0.170	141	0.910	149	0.115	109	0.510	110

TABLE II

Acidity increase in 1 ml. of digest at pH 8.6 (ml., 0.1 N NaOH)

Substrates / Time (hours) / Derivatives of aspartic acid in about 0.02 M final concentration	Triacetin				Butyl-Butyrate			
	1		24		1		24	
Control (No addition)	0.120	Degree 100	0.610	Degree 100	0.105	Degree 100	0.460	Degree 100
L-Aspartic acid	0.162	135	0.920	151	0.109	104	0.501	109
L-Benzoyl-aspartic acid	0.094	78	0.530	87	0.080	76	0.345	82
Succinic acid	0.122	101	0.610	100	0.105	100	0.456	99

alkaline to pH 9.2 with 1 N NaOH and kept at 37° as above. After 24 hours' incubation, 1 ml. of 0.1 M L-aspartic acid was added to the incubated glycerol-water extract.

These 3 ml. of esterase solutions, treated with alkali alone or with and without L-aspartic acid, were adjusted to pH 8.6 with 1 N HCl, mixed with 2 ml. of NH_4Cl-NH_4OH buffer containing 0.17 g. of triacetin (pH 8.6) and further incubated under toluene at 37° for 2 and 24 hours.

Control with the original esterase extract without alkali treatment.

Blanks without the enzyme or without the substrate. Values corrected for blanks are given as the acidity increase in Table III.

TABLE III

Acidity increase in 1 ml. of digest (triacetin) at pH 8.6 (ml., 0.1 N NaOH)

L-Aspartic acid added in final 0.02 M concentration \ Time (hours)	2		24	
Control Original enzyme without alkali treatment (No addition)	0.310	Degree 100	0.620	Degree 100
1) Incubation for 24 hours at pH 9.2 without addition	0.310	100	0.620	100
2) Incubation with L-aspartic acid for 24 hours at pH 9.2	0.397	128	0.917	148
3) After incubation for 24 hours at pH 9.2, L-aspartic acid is added	0.394	127	0.905	146
Incubation for 24 hours at pH 11.0 without addition	0.310	100	0.614	99

Activating Effect of L-Histidine on Olive Oil Hydrolysis by the Liver Esterase at pH 8.6.—Liver esterase extract: 2 g. of liver acetone powder, prepared as in the previous tests, were transferred into 50 ml. of 80% glycerol-water, rendered alkaline with 1 N-NH_4OH to pH 8.6 and incubated by stirring under toluene at 37° for 4 hours.

By centrifugation, the supernatant fluid was separated for the enzymatic use.

Test solutions: 5 ml. of olive oil emulsoid* was mixed with 3 ml.

* 6.5 g. of olive oil were vigorously shaken for one hour with 160 ml. of 1% polyvinyl alcohol solution, producing about $M/17$ olive oil emulsoid calculated as triolein.

of 0.2 *M* NH_4Cl-NH_4OH buffer at pH 8.6, 2 ml. of liver extract and 2 ml. of 1% $CaCl_2$ solution. The final concentration corresponded to about 0.02 *M* olive oil and L-histidine.

Besides these solutions, the control without L-histidine and the blanks without the substrate or without the liver extract were prepared and incubated under toluene at 37° for 24 and 48 hours.

The acidity increase in 5 ml. of digest was recorded in Table IV.

TABLE IV

Acidity increase in 5 ml. of digest (olive oil) at pH 8.6 (ml., 0.1 *N* NaOH)		
Additions \ Time (hours)	24	48
Control (No addition)	0.021	0.046
L-Histidine (0.02 *M* final concentration)	0.113	0.325

RESULTS AND DISCUSSIONS

The author (*5*) has reported on the stimulating effect of amino acids and some organic acids on pancreas lipase. The esterase action of liver powder was also accelerated in about 50% degree by the amino acids, such as L-aspartic acid, L-glutamic acid, L-ornithine and L-histidine, while the stimulation by glycine and by L-leucine was weaker (Table I).

The effect of the amino acids on the breakdown of butyl-butyrate by liver esterase was less than 10%, denoting negligible value, as in case of pancreas lipase, which was hardly affected even on the addition of L-histidine (Table I).

Regarding the activating groups of L-aspartic acid on the triacetin hydrolysis, it was ascertained that succinic acid remained without effect, and that L-benzoyl-aspartic acid behaved without influence or with a slight retarding on liver esterase, while pancreas lipase was similarly stimulated by these derivatives (Table II).

The results suggest the significance of the amino group of L-aspartic acid in the activation of liver esterase, while in the case of pancreas lipase the carboxyl of asparagine seems to be dominant.

While pancreas lipase was remarkably inactivated by the alkali treatment and reactivated for the most part by the addition of the

amino acids, as described in the first paper of this study (*5*), liver esterase, extracted from the liver powder, still kept its original activity for triacetin hydrolysis after the alkali treatment at pH 9.2–11.0 and at 37° for 24 hours (Table III).

The stimulation of esterase action by L-aspartic acid was also recognized in the case of the alkali treatment. The stability of pig liver esterase against alkali might be pointed out as its distinction from the pancreas lipase.

It should be here cited the observations of Leovenhart (*7*), that liver esterase is fitting to the hydrolysis of the mono-alcohol ester, breaking down the ester by its amount only as half as that of the pancreas lipase, while the hydrolysis of olive oil was achieved by liver esterae of 1,600 times as much as pancreas lipase, which is suited for the splitting of the natural fats. It is evident from Table IV, that the liver extract is hardly able to attack olive oil, showing the hydrolysis value so slight as less than 0.1 ml. of 0.1 *N* NaOH, while on addition of L-histidine in a 0.02 *M* final concentration, the hydrolysis of olive oil by liver esterase appeared to a remarkable degree.

The fact must be here stressed that L-histidine, which exerts a comparatively weak accelerating influence on liver esterase for triacetin breakdown, shows a significantly activating effect on it for the olive oil hydrolysis. These results lead to the conclusion that the specificity of liver esterase, apparently not fitting to the hydrolysis of natural fats, might be only conditional, since olive oil seems to undergo hydrolysis with ease in the presence of L-histidine.

SUMMARY

1. Here is investigated the effects of amino acids, especially of L-histidine, on the liver esterase, extracted from acetone powder of pig liver.

2. The splitting of triacetin by liver powder extract is stimulated in a degree of about 50% by addition of the amino acids, such as L-aspartic acid, L-glutamic acid and L-histidine, while the hydrolysis of butyl-butyrate is hardly influenced by these acids, as shown in the experiments with the pancreas lipase.

3. L-Benzoyl aspartic acid and succinic acid, however, did not show any stimulating influence, while aspartic acid had a notable activating effect. It must be, therefore, pointed out that free amino

group of L-aspartic acid is significant in the acceleration of triacetin breakdown by liver esterase.

Here must be cited the author's observations (*5*), that pancreas lipase is, on the contrary, activated by succinic acid.

4. Liver esterase appeared to be resistant to the alkali treatment at pH 9.2–11.0 and at 37° for 24 hours, whilst pancreas lipase is unstable in the presence of alkali.

5. It is very interesting to note that the liver enzyme, which was extracted from the acetone powder and showed negligible values of olive oil hydrolysis, becomes activated for olive oil splitting in the presence of L-histidine, showing its conditional specifity for the natural fats.

The author wishes to express his gratitude to Prof. Dr. Senji Utzino for suggesting this study and his guidance throughout the course of this research. These investigations owe much to the Ministry of Education for its Grants for the Scientific Researches, for which the author wishes here to express his deep appreciation.

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The Journal of Biochemistry, Vol. 38, No. 4.

STUDIES ON LYSINE AND ITS DERIVATIVES I. THE ASYMMETRIC ANILIDE SYNTHESIS OF LYSINE DERIVATIVES BY THE ACTION OF FICIN

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(Received for publication, July 26, 1951)

It has been reported (*1*) that papain as well as other papainase are capable of synthesizing CO-NH linkages, especially, so in terms of antipodal specificity. It therefore seems desirable to see whether ficin, a proteolytic enzyme of papain type, is capable of synthesizing peptide bonds.

The author has now succeeded in the enzymatic synthesis of anilide or phenylhydrazide when, in the presence of ficin, the acylated amino acid is incubated with aniline or phenylhydrazine at pH 4.5 and 37°.

The optimum pH of the enzymatic formation of hippurylanilide has been found to be close to 4.5, at which ficin is favorably able to perform hydrolysis (*2*).

The author has reported (*3*) that the antipodal specificity of ficin as a synthesizing enzyme is revealed when acyl derivative of DL-phenylalanine is incubated with aniline and ficin. On account of every slight solubility of the anilide formed, the antipodal specificity of ficin can be utilized as a method to resolve racemic amino acids into optical components. Thus DL-methionine and DL-lysine are resolved with ease by means of asymmetric enzymatic synthesis with ficin.

EXPERIMENTAL

Ficin Preparation—A mixture of one volume of the sap of *Ficus carica* and two volumes of 0.1 *M* citrate buffer at pH 4.5 was repeatedly filtered by suction until it became clear. A white curdy mass was precipitated when 3 volumes of acetone were added to the filtrate. The acetone precipitate was filtered by suction, washed with acetone and then with a small amount of ether. After evaporation of ether at room tempera-

ture it was dried in a vacuum desiccator over calcium chloride. The ficin preparation was obtained in pale brown powder, which was soluble in water. The yield amounted to about 11 per cent of the sap.

Synthesis of Hippurylanilide and its pH Optimum—0.9 g. of hippuric acid and 1 g. of aniline were added to a mixture of 40 ml. of citrate buffer of pH 4.5 and 420 mg. of ficin. The mixture was incubated at 37°. After about 10 minutes the anilide began to precipitate. Its amount was 557 mg. after 4 days, corresponding to 44 per cent of the theory. The substance recrystallized from alcohol melted at 212.5°. Calculated N 11.0, found N 10.9.

The yields of hippurylanilide were observed at several points of pH values. Fig. 1 shows the pH dependence of the enzymatic synthesis of the anilide. Its optimum has been found to be at pH 4.5.

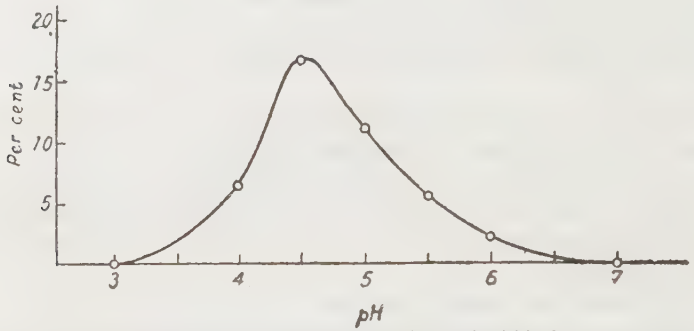

FIG. 1. pH dependence of synthesis of hippurylanilide by ficin. 0.9 g. of hippuric acid, 1 g. of aniline, 110 mg. of ficin; enough 0.1 *M* citrate buffer to make a total volume of 50 ml. were added; incubation temperature, 37°. The open circle represents the amount of anilide isolated after 24 hours, expressed in per cent of the theoretical maximum.

Resolution of DL-*Methionine with Ficin*—Benzoyl-DL-methionine was prepared from DL-methionine by the method of Carter (*4*) and recrystallized from 33 per cent alcohol. The yield amounted to 85 per cent of the theory; m.p., 151–152° (*5*).

To 8.4 g. of benzoyl-DL-methionine dissolved in 33 ml. of *N* NaOH, 3 g. of aniline and enough 0.1 *M* citrate buffer of pH 4.5 to make a total volume of 300 ml. were added. The mixture was kept at 37° with 1.7 g. of ficin. After 24 hours the benzoyl-L-methionine anilide which precipitated was recrystallized from ethyl acetate. Yield, 5.1 g. (95%); m.p., 160°.; calculated N, 8.5, found N, 8.2.

Four g. of benzoyl-L-methionine anilide were refluxed for 14 hours with 60 ml. of 6 *N* HCl. The hydrolysate when cold was filtered, the filtrate being concentrated *in vacuo* to a small bulk and then extracted with two 60 ml. portions of ether. The aqueous layer was again concentrated *in vacuo* almost to dryness. The residue was taken up in a minimum amount of absolute alcohol and adjusted to pH 5 with pyridine. The L-methionine which separated was recrystallized from 75 per cent alcohol. Yield, 1.3 g. (72%); calculated N, 9.4; found N 9.5; $[\alpha]_D^{25} = +20.5°$ (1% in 0.2 *N* HCl), which is practically identical with the value at 25° reported by Dekker and Fruton in the resolution of DL-methionine by papain (*5*).

The filtrate of the anilide was concentrated *in vacuo* to a small bulk and acidified with HCl to pH 2. The crystals which separated out were recrystallized from hot water. 1.2 g. of benzoyl-D-methionine were refluxed for 14 hours with 34 ml. of 6 *N* HCl. The hydrolysate was treated in the same fashion as for the L-isomer. Yield, 0.47 g. (66%); calculated N, 9.4; found N, 9.3; $[\alpha]_D^{20} = -19.3°$ (1% in 0.2 *N* HCl). The value at 20° for the recrystallized D-methionine reported by Dekker and Fruton was −15.7° (*5*).

Resolution of DL-Lysine with Ficin—One of the important factors controlling the enzymatic anilide synthesis is the nature of the racemic N-acylated amino acids. Accordingly, various ε-acylamino derivatives of *n*-caproic acid and α-acyl derivatives of ε-benzoyl-DL-lysine were prepared and subjected to the action of ficin; results are presented in Table I. Only α, ε-dibenzoyl-L-lysine reacted rapidly and quantitatively at pH 4.5, but the other compounds were unreactive. The data in Table I are fundamental to an enzymatic resolution of lysine. Recently, from the study of the nature of acyl groups, Doherty and Popenoe (*6*) recommended the diisobutyryl derivative of racemic lysine for the enzymatic resolution with papain.

Six g. of α,ε-dibenzoyl-DL-lysine were dissolved in 17 ml. of *N* NaOH and 6.3 g. of aniline and 280 ml. of 0.1 *M* citrate buffer at pH 4.5 were added. The mixture was incubated at 37° for 24 hours with 1.5 g. of ficin. The precipitated α, ε-dibenzoyl-L-lysine anilide was collected and recrystallized from alcohol. Yield 2.9 g. (80%); m.p., 214–215°; calculated N, 9.8, found N, 9.9.

In the isolation of L-lysine dihydrochloride, essentially the procedure of Eck and Marvel (*7*) was used. 4.5 g. of α,ε-dibenzoyl-L-lysine anilide were refluxed for 10 hours with 50 ml. of 6 *N* HCl. When cold

benzoic acid was removed by filtration. The filtrate was concentrated *in vacuo* to a thick syrup. It was dissolved in about 4 volumes (18 ml.) of hot absolute alcohol in a beaker. The solution was cooled to about 20°, and 5 volumes (23 ml.) of acetone were added slowly with stirring. L-Lysine dihydrochloride was filtered off, washed with ether, and dried. Yield, 1.9 g. (82%); m.p., 199–201°; calculated N, 12.8, found N, 12.6; $[\alpha]_D^{23}=+15.5°$ (3% in water).

After deproteinization by boiling, the filtrate of the anilide was treated in substantially the same manner as for the isolation of the D-isomer from the benzoyl-D-phenylalanine already reported (*3*). Yield, 0.94 g. (74%); m.p., 199–201°; calculated N, 12.8, found N, 12.9; $[\alpha]_D^{23}=-15.6°$ (3% in water). Berg (*8*) who employed D- and L-camphoric acids gave for the L- and D-isomers of lysine dihydrochloride $[\alpha]_D^{20}=+15.63°$ and $[\alpha]_D^{20}=-15.65°$ for 3 per cent solutions in water, respectively.

Table I

Synthesis by Ficin of L-Anilides from Acylderivatives of Lysine and its Related Amino Acids at pH 4.5

0.0025 *M* of substrate, 2.5 ml. of *N* NaOH, 0.01 *M* aniline, 55 mg. of ficin. The total volume was made up to 40 ml. with 0.1 *M* citrate buffer at pH 4.5; incubation temperature, 37°; incubation time, 24 hours.

Substrate	Yield of L-anilide
ε-Formylamino-*n*-caproic acid*	0 *per cent*
ε-Acetamino-*n*-caproic acid	0
ε-Chloroacetylamino-*n*-caproic acid	0
ε-Benzoylamino-*n*-caproic acid	0
ε-Benzoylamino-DL-α-bromo-*n*-caproic acid	0
ε-Benzoyl-α-formyl-DL-lysine	0
ε-Benzoyl-α-acetyl-DL-lysine	0
ε-Benzoyl-α-chloroacetyl-DL-lysine	0
α, ε-Dibenzoyl-DL-lysine	99

* ε-Formylamino-*n*-caproic acid (m.p., 89–90°; calculated N, 8.8; found N, 8.7) and ε-benzoyl-α-formyl-DL-lysine (m.p., 145–146°; calculated N, 10.1; found N, 10.2), as well as ε-acetamino-*n*-caproic acid (m.p., 105–106°; calculated N, 8.1, found N, 8.2) and ε-benzoyl-α-acetyl-DL-lysine (m.p., 144–145°; calculated N, 9.6; found N, 9.5), and ε-chloroacetylamino-*n*-caproic acid (m.p., 83–84°; calculated N, 6.8; found N, 6.9) (*9*) and ε-benzoyl-α-chloroacetyl-DL-lysine (m.p., 136–138°; calculated N, 8.6; found N, 8.6) were prepared from ε-amino-*n*-caproic acid and ε-benzoyl-DL-lysine according to the procedures of du Vigneaud and Irish (*10*), of Steiger (*11*) and of Fischer and Schoeller (*12*), respectively.

SUMMARY

1. In the presence of ficin, hippuric acid can be transformed with aniline or phenylhydrazine into its anilide or phenylhydrazide. The optimum pH of the enzymatic synthesis of hippurylanilide was found to be at 4.5, which is practically in agreement with the pH favorable to the hydrolysis of proteins by ficin.

2. The L- and D-isomers of methionine (+20.5° and −19.3°) and lysine dihydrochloride (+15.5° and −15.6°) were obtained by the asymmetric enzymatic synthesis of the N-benzoylated derivatives of the respective racemic amino acids.

3. The ε-formylamino, ε-acetamino, ε-chloroacetylamino and ε-benzoylamino derivatives of *n*-acproic acid, as well as ε-benzoyl-amino-DL-α-bromo-*n*-caproic acid and α-formyl, α-acetyl and α-chloroacetyl derivatives of ε-benzoyl-DL-lysine do not react with aniline at pH 4.5 under the influence of ficin.

The author is indebted to Prof. S. Utzino for his helpful criticism and guidance in the course of this study.

This work was aided by a Grant from the Ministry of Education for Scientific Research.

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The Journal of Biochemistry, Vol. 38, No. 4.

HYDROLYSIS OF ARGININE BY STREPTOCOCCUS FAECALIS*

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(Received for publication, August 18, 1951)

In 1940 Hills (*1*) reported that *Str. faecalis* contains an enzyme, arginine dihydrolase, which is different from the ordinary arginase. This enzyme was reported to hydrolyse L-arginine into ornithine and unstable tautomeric urea, which decomposed by itself spontaneously to ammonia and carbonic acid. According to Gale (*2*) the optimum of the faecalis-arginase was pH 5.5. These reports attracted our interest, since the specificity of arginase has been one of the subjects of studies in this institute.

A strain of *Str. faecalis* was isolated from human stool and cultivated on arginine containing slant agar broth media. In enzymatic experiments, the quantity of arginine remaining after action of bacteria was determined by flavianic acid method of Iwabuchi (*3*), modified by one of us (*4*). Decomposition of arginine was maximal at pH 7, and this optimum was quite different from pH 9 of arginase, heteroarginase (*5*), (*6*), and agmatinase (*6*), respectively. Liberation of ammonia was observed, and its quantity was not increased even after treatment of test solutions with soy bean urease. As no urease was found a priori in the bacteria, as Hills mentioned, our results seem to support his view of existence of a special arginine-dihydrolase, but when we examined our data, we noticed that the quantity of the ammonia formed was much less than that of disappeared arginine, calculated on the basis of Hills' view that 2 moles of ammonia should be produced per 1 mole of arginine. Therefore citrulline must have been produced intermediately. Indeed we could isolate 0.6 g. of citrulline copper salt (m.p., 257°) from the digest solution of 2.1 g. of arginine hydrochloride.

Enzymatic formation of citrulline from arginine has been known since Ackermann (*7*), Horn (*8*) and Tomota (*9*), but the yields reported by them were not more than 10%. Horn named the res-

* A part of contents of "The specificity of enzyme action," spoken at the 12th General Japanese Medical Congress, 3 April 1947, Osaka Municipal Auditorium, Osaka.

ponsible enzyme arginine desimidase. Since, however, the actual nature of the chemismus must be deamidation of guanidine residue and successive tautomery of the formed —C(=NH)-OH radical to -CO-NH_2, we prefer a provisional nomenclature "metarginase" than the misleading name of "arginine deamidase" and want to distinguish the enzyme from other guanidine decomposing enzymes such as arginase, heteroarginase and agmatinase.

Our experiment with an increased quantity of bacterial suspension showed on the other hand that no arginine was left, whereas ammonia formation was over 1 mole. *Str. faecalis* must, therefore, contain a citrulline decomposing enzyme, citrullinase. For studies of this enzyme we prepared citrulline (m.p. 281°, $[\alpha]_D^{\alpha}=+3.59$) chemically out of arginine according to Hunter and Gornal (*10*), because the above mentioned copper salt at our hand was not much enough for further experiments. Ammonia production from citrulline by *Str. faecalis* was optimal at pH 7, as in the case of metarginase. Citrullinase does not exist in liver, lung, kidney, muscle, testis of rabbit and also not in *Bac. coli communis*. By cultivating bacteria on citrulline broth media, *etc.*, we endeavoured to obtain the strains of faecalis which might hydrolyse citrulline intensively, but none of them was found to produce in enzymatic tests any more than 18% ammonia under the condition of toluene addition. At any rate the cause of our detection of metarginase in *Str. faecalis* may be due to the fact that our strain does not contain a strong citrullinase, and we may perhaps say that the arginine dihydrolase of Hills was but a mixture of metarginase and citrullinase.

As above mentioned the pH optima of metarginase and citrullinase are both 7, while according to Gale his faecalis arginase acted optimally at pH 5.5. However, Gale has measured only CO_2, not ammonia, production from arginine. To explain the disagreement of pH optima we cultivated *Str. faecalis* in glucose broth to which this time no arginine was added.

These bacteria were inactive on arginine, *i.e.*, 100% arginine remained unattacked, while they liberated CO_2 from citrulline. Optimum was pH 4. Ammonia production could not be proved by soda-Folin method. Therefore *Str. faecalis* cannot decarboxylate arginine, but they decarboxylate citrulline. If metarginase and citrulline carboxylase would act together upon arginine, the maximal CO_2 production might be observed in the intermediate acidity between pH 7 and 4.

Str. faecalis does not form ammonia out of D-arginine, L-arginic acid,

glycocyamine and hydantoic acid in the same condition as for arginine decomposition.

EXPERIMENTAL

A strain of *Str. faecalis* was isolated from stool of children, cultivated 24 hours on slant arginine broth agar media, washed 2 times with distilled water, and suspended in 3 ml. of water per one test tube of culture media and mixed together. In one experiment 1 ml. of suspension contained 4.6 mg. of dried bacterial bodies. In another experimental series the weight of dried bacteria was not measured each time, but they were not much different from the value given above if not otherwise mentioned.

Determination of Arginine—The method of Iwabuchi was modified to accelerate the crystallization of arginine flavianate (*4*). To 4 ml. of test solution were added 1 ml. of 10% lead subacetate and 5~10 minutes later 1 ml. of 10% sodium nitrate. 3 ml. of the filtrate were neutralized to congo red paper with 1 ml. of 1.5% sulfuric acid and filtered. Through this deproteinizing process the original test solution was diluted to 1:2. To 1 ml. of this filtrate was added 1 ml. of 3% flavianic acid. Precipitation of arginine flavianate occured immediately. Colorimetry of the flavianic acid in the precipitate was done according to Iwabuchi.

Hydrolysis of Arginine by Streptococcus faecalis—2 ml. of $N/10$ arginine hydrochloride + a ml. of $N/10$ NaOH (for adjustment of pH of arginine solution) + $(4-a)$ ml. of water + 2 ml. of $M/10$ buffer + 2 ml. of bacterial suspension + 0.5 ml. of toluene.

	Arginine disappeared				
	pH 5	pH 6	pH 7	pH 8	pH 9
After 2 hours	13.6 %	21.3 %	27.5 %	25.4 %	16.7 %
„ 24 „	42.9	61.8	84.0	80.1	50.5

Quantitative Relationship between the Arginine Disappeared and the Ammonia Formed.—Composition of test solutions was the same as above except that a more concentrated suspension was used. Ammonia was determined before and after soy bean urease action (pH 7) by 50°-soda-Folin method. 100% of ammonia in the following table mean a formation of 2 moles of ammonia from 1 mole of arginine.

pH	Time	NH_3 formed		Arginine disappeared
		After urease	Before urease	
7	2 *hours*	43.6 %	42.7 %	61.3 %
	24	66.0	67.2	100
9	2	29.1	28.5	41.5
	24	45.5	46.3	95

In the same condition of 24 hours test (pH 7) bacteria liberated only 0.8% ammonia from urea, 0.4% ammonia from hydantoic acid, and 0~1% urea (determined by urease method) from D-arginine, L-arginic acid and glycocyamine.

Isolation of Citrulline from Arginine Hydrolysate—2.1 g. of arginine hydrochloride were dissolved in 50 ml. of *M*/10 phosphate. pH was 6.8. Bacteria suspension out of 10 slant broth (+arginine) agar media was added to it, the total volume was filled with water to 500 ml. and placed under toluene at 37°. In 4 days a test showed that no arginine remained. The solution was, therefore, deproteinized with 10% lead subacetate and pH was adjusted to 7 with NaOH. The centrifuged supernatant fluid was freed from lead with H_2S, concentrated in vacuum to a small volume, neutralized to litmus with 10% soda solution, mixed with 35% mercuric acetate, then with soda solution to bring slightly alkaline, further with ethanol to make the solution of 5% alcohol concentration, and placed for 24 hours in an ice box. The precipitate was washed with 80% alcohol, suspended in water, demercurized with H_2S, decolorized with active charcoal, concentrated in vacuum, diluted with 5% sulfuric acid and mixed with phosphotungstic acid to be freed from impurity. The filtrate was then freed from sulfuric acid and phosphotungstic acid, decolorized and concentrated in vacuum to 50 ml. To this solution was added copper hydroxide, which was prepared from 12.5 g. copper sulfate, and filtered after 15 minutes heating. The violet blue colored solution was placed in an ice box and 0.6 g. of copper salt of citrulline was obtained. m.p. 257°. Found N, 19.9% (calculated N, 20.4%).

Citrullinase of Str. faecalis.—2 ml of *M*/10 citrulline were used, in other points the composition of test solutions was the same as in the ex-

Hydrolysis	pH 5	pH 6	pH 6.6	pH 7	pH 7.4	pH 8	pH 9
After 2 hours	4 %	5.6 %	8.4 %	9.0 %	8.0 %	5.4 %	2.2 %
» 24 »	10.6	9.0	15.4	17.0	12.2	6.6	4.2

periment on metarginase. Ammonia determination according to 50%-soda-Folin.

Non Existence of Arginine Carboxylase in Str. faecalis—Bacteria were cultivated in 10 slant broth agar media, transferred to 1 *l.* of glucose (2%) broth media (pH 5), and after 20 hours centrifuged. The precipitated bacteria were twice washed with water and suspended in 16 ml. of water. The composition of test solutions was the same as in the case of metarginase, except that 0.5 and 0.2 ml. of *M*/10 HCl were used in pH 3 and 4 tests, respectively, instead of *a* ml. of NaON. Remaining arginine was determined with liver arginase (pH 9)-soy bean urease (pH 7) method. Our liver arginase solution, which was prepared through autolysis and dialysis, is inactive upon agmatine. The percentage given in the next table means the amount of remaining arginine.

	Arginine remained			
	pH 3	pH 4	pH 5	pH 6
After 2 hours	97.8 %	100.2 %	100.2 %	100.6 %
„ 6 „	97.6	99.6	99.4	100.2

Citrulline Carboxylase and its pH Optimum—The composition of the test solution was the same as in the previous experimental section, except that 1 ml. of 1 *M* citrulline was used. CO_2 production was measured in Kobayashi's apparatus (*11*).

After hours	1/2	1	2	4	6
pH 3	0.15 *ml.*	0.19 *ml.*	0.40 *ml.*	0.49 *ml.*	0.63 *ml.*
4	0.37	0.58	1.09	1.55	1.94
5	0.25	0.34	0.62	0.73	0.88
6	0.03	0.08	0.17	0.23	0.32

At pH 4 decarboxylation was 7.6% after 6 hours. At the end of CO_2 measurement, 5 ml. of each test solution were analysed for free ammonia with 50%-soda-Folin method, but no ammonia was found in all cases.

SUMMARY

1. Strains of *Streptococcus faecalis*, which have been cultivated upon arginine broth agar media, contain an enzyme, metarginase. This enzyme

hydrolyses L-arginine into citrulline+ammonia. pH optimum is 7. Citrulline can be isolated as copper salt with a good yield.

2. Metarginase is inactive upon D-arginine, L-arginic acid and glycocyamine.

3. The bacteria tested contain also citrullinase, which hydrolyses citrulline into ornithine NH_3+CO_2. pH optimum is 7. This enzyme is inactive upon hydantoic acid.

4. Citrullinase is not found in animal organs and *Bac. coli communis*.

5. Arginine can be hydrolysed to ornithine+2 NH_3+CO_2 through the combined action of metarginase and citrullinase.

6. *Str. faecalis*, cultivated in glucose broth, decarboxylates citrulline without formation of ammonia. pH optimum of citrulline carboxylase is 4.

Expenses of this work were defrayed by Grants for the Advancement of Science from the Department of Education, Japan.

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The Journal of Biochemistry, Vol. 38, No. 4.

STUDIES ON SPECIFICITY OF HETEROARGINASE*

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(Received for publication, August 18, 1951)

According to our studies on the guanidino group splitting enzyme system, the ordinary arginase attacks only natural L-arginine, but there is in *Streptococcus faecalis* another enzyme, metarginase (*1*), which also hydrolyses L-arginine specifically, yielding citrulline and ammonia as reaction products. On the other hand agmatine, which is the decarboxylated arginine, is transferred by hydrolytic action of a specific enzyme, agmatinase (*2*), into putrescine and urea. These enzymes are, however, quite different from heteroarginase (*2, 3*), which produces urea from D-arginine, L-arginic acid, acyl L-arginine and δ-guanidino valeric acid.

We studied this time about susceptibility to hydrolysis of ω-guanidino fatty acids in respect to their C atom number of fatty acid residues. The substrates examined were ε-guanidino caproic acid, δ-G valeric acid, γ-G butyric acid, β-G propionic acid and G acetic acid, namely glycocyamine.

The solution of heteroarginase used in the present experiment was a simple extract of rabbit intestinal mucous membrane with cane sugar solution, since though according to Sano (*3*) this extract contained arginase and heteroarginase, we have confirmed that the arginase itself, which is prepared from rabbit liver through autolysis and dialysis, is inactive upon D-arginine and the above mentioned guanidino fatty acids. The heteroarginase solution was able to hydrolyse D-arginine in higher degree than L-arginine. In experiments with this extract urea production from the guanidino compounds was measured by the urease method. Results are shown in Table I.

Hydrolysis of L-arginine should be attributed, of course, to the contaminated ordinary arginase. In this table, the point of interest is that δ-G valeric acid, C atom number of which is the same as that of

* A part of contents of "The specificity of enzyme action," spoken at the 12th General Japanese Medical Congress, 3 April 1947, Osaka Municipal Auditorium, Osaka.

TABLE I

Action of Heteroarginase at pH 9

Substrate (*M*/50)	Hydrolysis	
	After 2 hours	After 24 hours
ε-Guanidino caproic acid	2.0 %	3.6 %
δ- " valeric acid	27.0	41.4
γ- " butyric acid	28.0	71.5
β- " propionic acid	0	0
Glycocyamine	0	0
D-Arginine	60.5	90.4
L-Arginine	31.5	70.6

arginine, and γ-G butyric acid, which may be expected as an oxidative deamination and successive decarboxylation product of that amino acid, are split to a good extent, whereas the hydrolytic per cent of ε-G caproic acid remains so small as to be regarded as a value lying within the limits of experimental error.

The pH optimum of δ-G valeric acid hydrolysis was 9, as shown in Table II.

TABLE II

Hydrolysis of G Valeric Acid

pH	After 2 hours	After 24 hours
6	4.9 %	39.8 %
7	20.9	45.4
8	42.6	70.5
9	58.1	75.9
9.6	57.0	73.0

The physiological meaning of heteroarginase contained in fresh liver, but not in its autolysate has been interpreted by Nakamura (*2*) to the effect that it affects the guanidino group of arginine residue, when this amino acid is located at the end of polypeptide chain. The significance of this enzyme may also be sought in the point that if γ-G butyric acid would be produced from arginine, it could further be transferred to β-hydroxy-γ-amino butyric acid, namely carnithine.

β-Oxidation of γ-G butyric acid itself to glycocyamine must be denied, because arginine cannot be the mother substance of creatine, as already clearly proved by American authors.

Whether or not the above mentioned guanidino fatty acids can be split enzymatically, has been a problem for a long time. According to Thomas (*4*) γ-G butyric acid and β-G propionic acid were hydrolysable by the action of liver press juice whereas ε-G caproic acid was not; the hydrolysis of γ-G butyric acid was, however, denied afterwards by himself (*5*). According to Edlbacher (*6*), and Edlbacher and Bonem (*7*) β-G propionic acid and glycocyamine were resistent against extract of dried liver powder. Dakin (*8*) reported also unhydrolysability of glycocyamine through liver enzyme, but according to Karashima (*9*) and Felix and Schneider (*10*) it could be attacked by liver brei or its glycerol extract. Concerning δ-G valeric acid, Hellermann *et al.* (*11*) reported at first its innertness against the action of liver arginase, but later they (*12*) (*13*) asserted that it could be attacked by the same enzyme.

Our experiments have shown that glycocyamine is not hydrolysed by intestine heteroarginase in test solutions of various pH. Next we tested whether or not the extract with cane sugar solution of guinea pig liver could hydrolyse that compound and indeed we observed a slight positive result at pH 7–9, as shown in Table III.

TABLE III

Hydrolysis of Glycocyamine

pH	After 2 hours	After 24 hours
6	0 %	0 %
7	1.6	2.6
8	0.4	4.2
9	2.9	4.6
9.6	0.5	0

These values were calculated from the differences in ammonia production between the main and control tests. The figures obtained for the 24-hour-incubation samples were not large, so that we think it relevant to reserve, for the time being, the assertion of possible cleavage of glycocyamine by the enzyme in question.

EXPERIMENTAL

Preparation of Substrates—ω-Amino acids were prepared at first in the following ways: ε-Amino caproic acid (Organic Syntheses XVII, 7); δ-Amino valeric acid (see Nakamura (*2*); γ-Amino butyric acid was obtained by biological decarboxylation of glutamic acid.

Bact. coli communis were cultivated according to Gale (*14*) 18 hours in glucose (2%) broth media, washed three times with water, suspended in 600 ml. of water, poured into 2 *l.* of glutamic acid solution, which had been neutralized with sodium hydroxyde and then acidified to pH 5 with acetic acid, and the mixture was incubated 7 days at 37°, when the specific taste of the amino acid disappeared. Then centrifuged supernatant fluid was adjusted to pH 5 with sodium hydroxyde, heated, decolorized and dried under diminished pressure. The residue was suspended in 200 ml. of ethanol, esterified by passing dried hydrochloric acid gas, freed from insoluble mass, concentrated in vaccum to 25 ml., diluted with 100 ml. of water and boiled to hydrolyse the formed ester. From this solution chlorine ion was removed by adding an excess of silver oxide, dissolved silver was then removed with H_2S and the filtrate was concentrated in vacuum to 10 ml. and mixed with 100 ml. of ethanol. Rubbing the cooled glass wall generated crystallisation of free amino butyric acid, quantity of which increased after storage in ice box for three days. m.p., 197°. The yield was 5.6 g.

β-Amino propionic acid (Organic Syntheses XVI, 1); Guanidination of ω-amino acids was done according to Kapfhammer and Müller (*15*). ε-G caproic acid (1.7 g. from 2 g. of ε-amino caproic acid); δ-G valeric acid (1.6 g. from 2.25 g. of δ-amino valeric acid); γ-G butyric acid (1.32 g. from 2 g. of γ-amino butyric acid); β-G propionic acid (2 g. from 2 g. of β-amino propionic acid); Glycocyamine: according to Ramsay (*16*).

Preparation of Enzymatic Test Solution—Guanidino fatty acids are in general slightly soluble in water. Preparation of $M/20$ solutions of G caproic acid, G valeric acid and glycocyamine was as follows 1 millimole of each compound was weighed, dissolved in 10 ml. of $N/10$ HCl, diluted with water to double volume. 4 ml. of $M/20$ substrate solution +1 ml. of $N/5$ NaOH+2 ml. of $M/10$ of glycine buffer+1 ml. of $M/10$ $MnSO_4$+2 ml. of enzyme solution. $M/25$ solutions of G propionic acid and butyric acid were prepared by dissolving 1 millimole of them in hot water and after cooling the supersaturated solutions diluted to 25 ml., 5 ml. of which were used instead of 4 ml. of substrate solution+

+1 ml. of NaOH in the above mentioned test solutions. In the case of L- or D-arginine, 2 ml. of $M/10$ solution of those hydrochloride + 1 ml. of $N/10$ NaOH+2 ml. of water=5 ml. were used. The final concentration of substrates was $M/10$ in all cases. Toluene was added as antisepticum.

Determination of urea was conducted by the urease method (pH 7) and 50°-soda-Folin distillation method. In experiments to determine the pH optimum, $M/10$ phosphate buffers were used.

Enzyme Solutions—(A) Heteroarginase: Intestinal mucous membrane brei of rabbit was added to 5 volumes of 10% cane sugar solution, stored under toluene 2–3 days in ice box and the centrifuged supernatant was used as enzyme solution. (B) Arginase: Liver brei of rabbit was autolysed 3 days at 37° with 5 volumes of water in antiseptic condition (with toluene addition), filtered and dialysed against water.

Results, which were not stated in the text, are presented here.

Hydrolysis by Arginase (pH 9)

	After 2 hours	After 24 hours
L-Arginine	73.0 %	96.4 %
D-Arginine	1.0	0.5
ε-G caproic acid	2.5	1.6
δ-G valeric acid	0	0.7
γ-G butyric acid	2.1	1.2
β-G propionic acid	2.8	4.0
Glycocyamine	0	1.0

A slight cleavage of propionic acid derivative *etc.* may be due to experimental error.

SUMMARY

Among various ω-guanidino fatty acids the substrates undergoing hydrolysis by heteroarginase were found to be δ-guanidino valeric acid and δ-guanidino butyric acid. The corresponding derivatives of caproic, propionic and acetic acid, namely glycocyamine, were refractory to the action of the enzyme. Physiological meaning of heteroarginase was discussed.

Expenses of this work were defrayed by Grants for the Advancement of Science from the Department of Education, Japan.

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The Journal of Biochemistry, Vol. 38, No. 4.

STUDIES ON THE AGGLUTINATING AND HEMOLYSING FACTORS CONTAINED IN RICINUS COMMUSIS. I. STUDIES ON THE AGGLUTINATING FACTOR

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(Received for publication, August 1, 1951)

It has been well known that various plant organs, such as seeds of *Aburus precatorius* or *Ricinus communis*, which, like agglutinins or hemolysins in the serum of immunized animals, agglutinate or hemolyze erythrocytes of certain animals. The agglutinating substance in *Ricinus communis* is soluble in alkali and unstable against the heat (*4*), and considered to be ricin by many investigators (*1*, *2*, *3*), and some of them (*5*) supposed that the toxicity of ricin may be due to its agglutinating property. The purpose of the present studies is to make clear the chemical nature of the agglutinating substance in *Ricinus communis*.

EXPERIMENTAL

The extract of Ricinus communis was diluted with saline solution 10, 20, 50, 100, 200, 500, 1000, and 2000 times, and to each 0.5 ml. of these diluted solutions two drops of 10 per cent suspension of pig erythrocytes were added and after an incubation for 1 hour at 37° with occasional shaking the degrees of agglutination were determined. In recording the results, the cases where no agglutination occurred are designated by (—), and complete agglutination by (卌). For intermediate cases the signs (±), (+) and (卄) are used according to the grade of agglutination.

Extraction of the Active substance from Castor Bean—Castor bean were ground well and extracted with ether thoroughly in Soxhlet's apparatus. The defatted material was dried in vacuum and powdered completely and extracted with 100 times portion in weight of saline solution. The active substance was found only in the water extract, as shown in Table I.

The ethereal extract was evaporated to dryness and the residue was dissolved in a small amount of alcohol. To this alcohol solution 30 times portion in weight of saline solution was added under stirring

TABLE I

Agglutination Test with the Water Soluble and Insoluble Portions of Defatted Castor Bean and Ether Soluble Portion of Caster Bean

Degree of dilution of the solution	10	20	50	100	200	5000	1000	2000
Water soluble portion	##	##	##	##	##	##	##	##
Water insoluble portion	—	—	—	—	—	—	—	—
Ether soluble portion	—	—	—	—	—	—	—	—

and an emulsion was obtained. After 20 minutes the emulsion was submitted to the agglutination test. The water insoluble portion of the defatted powder was suspended in 100 ml. of saline solution and used for agglutination test.

The protein reactions suh as biuret-, xanthoprotein-, Millon's, Hopkins-Cole's, Molisch's, Pb-acetate-, and Heller's reactions were all positive with the water extract, and the precipitate was formed on adding sulphosalicylic acid, trichloroacetic acid or Almen's reagent. The water extract showed also positive reducing reaction by Fehling's Benedict's or Nylander's reagent after hydrolysis by heating with HCl.

The Effect of Proteinase and Amylase on the Active Substance—As it is certain that the active extract contains both protein and the reducing substance, likely to be carbohydrate, the experiments were performed to decide whether protein or carbohydrate itself possesses the agglutinating power. For this purpose the active extract of the defatted powder of ricinus was subjected to the action of pepsin or amylase and subsequently tested for its aggluting activity.

Pepsin was purified by Willstatter's method (*6*) until no more glycolytic action was observed. To 2 ml. of the saline extract of the defatted ricinus powder 1 ml. of 1 per cent solution of the purified pepsin was added together with HCl to adjust the pH to 1.8. The mixture was kept at 37° until protein reactions disappeared completely (Usually it took about 3 hours). For the control test the boiled pepsin solution was used. When digestion was complete the solution was neutralized with NaOH and diluted to 20 ml. with saline solution.

As amylase Taka-diastase (Commercial) was used. 0.4 ml. of 1 per cent solution of amylase was added to 2 ml. of the saline extract of defatted ricinus powder and digested at 37° for 1 hour. The control test was carried out with heated amylase solution.

TABLE II

Agglutination Test with the Castor Bean Extract Treated with Pepsin or Taka-amylase

Degree of dilution of the solution	10	20	50	100	200	500	1000	2000
Solution digested with pepsin	+	−	−	−	−	−	−	−
Solution treated with boiled pepsin	##	##	##	##	##	##	##	##
Solution digested with amylase	##	##	##	##	##	##	##	##
Solution treated with boiled amylase	##	##	##	##	##	##	##	##

As shown in Table II, the agglutinating substance in castor bean is readily destroyed by pepsin, but not by amylase.

Further Purification of the Active Substance by Adsorption Technique—The saline extract was acidified with HCl to pH 2.6 and the formed precipitate was removed by centrifugation. To the supernatant liquid alumina was added in a ratio of 1 g. to 10 ml., and the mixture was let stand for 1 hour at room temperature with occasional shaking. The centrifuged alumina was washed twice with physiological saline solution, in advance adjusted to pH 2.6 with HCl. The adsorbed substance was eluted with saline solution at pH 7.8–8.0. The eluate was neutralized with HCl. The precipitate formed by saturation with sodium sulphate was collected and dialyzed in a collodium sack against running water until it became free of sulphate ion reaction. This solution was concentrated to a half volume under reduced pressure by aerating

TABLE III

Agglutination Test with the Castor Bean Extract Treated with Alumina

Degree of dilution of the solution	10	20	50	100	200	500	1000	2000
Original saline solution	##	##	##	##	##	##	##	##
Precipitate at pH 2.6	+	−	−	−	−	−	−	−
Supernatant liquid at pH 2.6	##	##	##	##	##	##	##	##
Supernatant liquid treated with alumina at pH 2.6	+	±	−	−	−	−	−	−
Eluted solution at pH 7.8–8.0	##	##	##	##	##	##	##	##
Precipitate by alcohol at 0°	##	##	##	##	##	##	+	−

carbon dioxide slowly at the temperature below 40°, to which alcohol was then added to a concentration of 75 per cent, keeping the temperature below 0°. The precipitate was collected and freed from alcohol as quickly as possible and dried in vacuum. By these procedures a light yellow powder was obtained, which possessed a strong agglutinating power against pig erythrocytes. This preparation contained, on dry weight basis, 14.2 per cent nitrogen, and showed distinct protein reactions, but no reducing reaction at all, even after acid hydrolysis.

Some Properties of the Purified Agglutinating Substance—The influence of heating: one per cent solution of the preparation in physiological saline solution was heated at 60, 70, or 80°. for 30 minutes, and the respective agglutinating activity was examined. It was found that the activity was completely lost at 80° accompanying no coagulation (Table IV).

TABLE IV

Agglutination Test with the Preparation Heated at Various Temperatures

Degree of dilution	10	20	50	100	200	500	1000	2000
Original solution	##	##	##	##	##	##	+	−
Heated at 60°	##	##	##	##	##	##	+	−
Heated at 70°	##	##	##	##	##	+	−	−
Heated at 80°	−	−	−	−	−	−	−	−

The effect of acid and alkali: one per cent solution of the preparation in physiological saline solution was adjusted to various pH with HCl or NaOH. After standing for 1 hour at room temperature, the solutions were then neutralized with NaOH or HCl, respectively, and the changes in the agglutinating activity were observed, as shown in Table V.

The activity was almost completely lost at pH 9.6, but not affected at acid side at all.

(1) Precipitation of the substance by various salts: to the solution of the preparation ammonium sulphate or sodium chloride was added to 1/2, 2/3 or a complete saturation. After standing for 30 minutes the formed precipitate was collected respectively by centrifugation and dialyzed in a collodium sack against running water until no reaction of ammonia or chlorine was recognized. With these solutions the agglutination tests were performed (Table VI).

TABLE V

The Influence of Acid or Alkali

Treatment \ Degree of dilution	10	20	50	100	200	500	1000	2000
Original solution	##	##	##	##	##	##	+	−
Treated at pH 1.4	##	##	##	##	##	##	+	−
Treated at pH 3.4	##	##	##	##	##	##	##	−
Treated at pH 8.6	##	##	##	##	##	+	±	−
Treated at pH 9.6	##	−	−	−	−	−	−	−

TABLE VI

Agglutination Test with the Solution of the Preparation Treated with Ammonium Sulphate or Sodium Chloride at Various Concentration

Kind of salts \ Grade of saturation	1/2 saturation	2/3 saturation	Total saturation
Ammonium sulphate	+	##	##
Sodium chloride	−	−	−

The active substance was precipitated completely at 2/3 saturation of ammonium sulphate, but not by sodium chloride at any degree of saturation.

(2) The effect of formaldehyde and sodium nitrite: since the active substance seems to be of protein nature, it is worth while to examine the effect of formaldehyde or sodium nitrite that react with free amino group in protein molecule. (A) One per cent solution of the preparation in physiological saline solution was mixed with one per cent formaldehyde solution in a ratio of 4:1 and kept at room temperature for 30 minutes. The mixture was dialyzed in a collodium sack for removal of remaining formaldehyde. Sodium chloride was dissolved in it to a concentration of 0.85 per cent. (B) To 5 volumes of the one per cent solution of the preparation one volume of 30 per cent sodium nitrite solution was added and kept at room temperature for 30 minutes. The

remaining sodium nitrite was then removed by dialysis. From the results shown in Table VII, it seems very likely that the amino agroup in protein molecule plays an essential part in the agglutinating activity of the substance.

TABLE VII

Agglutination Test with the Solution of the Preparation Treated with Formaldehyde or Sodium Nitrite

Degree of dilution	10	20	50	100	200	500	1000	2000
Control test	##	##	##	##	##	##	+	−
Treated with HCHO	−	−	−	−	−	−	−	−
Treated with $NaNO_2$	−	−	−	−	−	−	−	−

(3) The effect of alcohol the activity at various temperature: in the preceeding experiment it has been demonstrated that the active substance can be precipitated without losing its activity by adding alcohol only at low temperature below 0°. To the solutions of the preparation ethanol was added to a concentration of 50 or 70 per cent, and kept at 0° or 18° for 30 minutes. The precipitates formed were then collected by centrifugation and freed from ethanol by dialysis at a temperature below 0°. Each fraction was dissolved in the same volume of saline solution and the agglutinating activity was determined.

TABLE VIII

Agglutination Test with the Solution of the Preparation Treated with Ethanol at Various Temperatures and Concentrations

Temperature	0°		18°	
Final concentration of ethanol *per cent*	50	70	50	70
Supernatant liquid	−	−	−	−
Precipitate	##	##	±	±

(4) Relation between agglutinating activity and various salts: the experiments were carried out to determine whether or not the agglutination would be due to the active substance appeared in the absence of sodium chloride. The preparation was dissolved in isotonic salts or

glucose solution, to which the suspension of erythrocytes prepared with the corresponding salt or glucose solution was added, and the agglutination tests were performed. From the results summarized in Table IX it may be seen that the agglutination is due to the active substance appeared in the media of various inorganic salts besides sodium chloride, but not in the medium replaced by glucose. The presence of salt seems to be necessary for the agglutination by the active substance.

TABLE IX

Effect of Various Salts and Glucose on the Agglutination Due to the Active Substance

Degree of dilution	10	20	50	100	200	500	1000	2000
KCl, 1.2%	###	###	###	###	###	###	##	—
$CaCl_2$, 1.2%	###	###	###	###	###	###	##	—
$MgCl_2$, 1.0%	###	###	###	###	###	###	+	—
Na_2SO_2, 1.9%	###	###	###	###	###	###	+	—
Glucose, 5.1%	—	—	—	—	—	—	—	—
Control (0.85% NaCl)	###	###	###	###	###	###	##	—

The Action of the Substance against Erythrocytes of Various Animals—In this section the experiments were carried out with erythrocytes of other species of animals than pig described hitherto. As shown in Table X erythrocytes of sheep and of horse were not agglutinated at all, and those of ox a little.

SUMMARY

A substance that agglutinates the erythrocytes of various kinds of animals has been found in castor bean. This substance was isolated and demonstrated the following properties:

1. When the substance is digested by pepsin, the activity is almost completely lost.

2. The substance is extracted with saline solution, adsorbed on almina, eluted at pH 7.8–8.0, and precipited by the addition of sodium sulphate or ethanol. Thus purified white powder contains 14.2 per cent of nitrogen.

TABLE X

Agglutination Test with the Active Substance and the Erythrocytes of Various Animals

Species of erythrocytes \ Degree of dilution	10	20	50	100	200	500	1000	2000
Human (A type)	##	##	##	##	##	##	##	—
" (B type)	##	##	##	##	##	##	##	—
" (O type)	##	##	##	##	##	—	—	—
Sheep	—	—	—	—	—	—	—	—
Guinea pig	##	##	##	##	##	—	—	—
Horse	—	—	—	—	—	—	—	—
Ox	##	+	—	—	—	—	—	—
Rabbit	##	##	##	##	##	##	—	—

3. The substance is inactivated completely by heating at 80° for 30 minutes.

4. The substance is stable against acid, but rather destroyed at alkaline reaction above pH 9.6.

5. The substance is precipitated by ammonium sulphate at 2/3 saturation, but not at 1/2 saturation.

6. The substance loses its activity completely by the treatment with formaldehyde or sodium nitrite.

7. The substance can be precipitated by 50 per cent ethanol below 0° without losing its activity, but at 18° the activity disappeares completely.

8. The agglutination appeares in the presence of inorganic salts, but not of glucose solution.

9. The substance agglutinates the erythrocytes of human, guina pig, and rabbit, but does not those of sheep and horse.

The author whishes to express his sincerest thanks to Prof. S. Fujimura for his kind advice and encouragement in carrying out this investigation.

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ABSTRACTS

from

The Journal of Japanese Biochemical Society (Seikagaku).
Volume 20 (1948) p. I-II.

1. The oxidation of 8-methylquinoline by puinoline dehydrogenase. Yukichi Hashimoto, p. 17. cf., **19**, 159 (1948). 8-Methylquinoline was found to be converted into 2-hydroxy-8-methylquinoline with 20% water exts. of rabbit liver at pH 7.0.

2. Microdetermination of blood phosphorus. Kishima Imai, p. 18–19. The author has been reported a colorimetric method for blood P detn., basing on the quantitative combination of methyleneviolet with phosphotungstate: To 2.0 ml. of dild. blood (100 times), add 2 ml. of 5% sulfosalicylic acid for the deproteinization. (A) Inorganic P—Transfer 2.0 ml. of the filtrate into a calibrated centrifuge-tube, add 4 ml. of reag.-mixt. of 4.2% amm. molybdate in 5 *N* HCl and of 1% methylene violet (1 : 3), and centrifuge 10 min. later. After washing the ppt. twice with each 4 ml. of 1 *N* HCl, add acetone to the 10 ml.-mark, and compare purple-red color against the stand, soln, (0.5 or 1.0 γ per ml.); (B) Acid sol. P—After digestion of 1.0 ml. of the filtrate with conc. HCl, run the procedure (A), and calcd. out acid sol. P. The results with author's method are satisfactory comparing with those by Fiske-Subbarow's method.

3. Vitamin B_1 tolerance experiments with human subjects. Tetsu Asari, p. 20–32. After the administration of vitamin B_1 ($V.B_1$) to healthy adults, the urinary excretions of $V.B_1$ have been studied: 5,000 γ of $V.B_1$, given *per os* and by injection, result the max. excretion after 1–2.5, and 1 hrs. respectively; daily excretion of $V.B_1$ in 24 hrs. urine are nearly constant even by the administration of 2,000–5,000 γ of $V.B_1$; successive administrations of 2,500–10,000 γ of $V.B_1$ for 2–3 weeks, do not appreciably change the daily excretion (1,000–1,800 γ $V.B_1$); pyrophophate ester of $V.B_1$ is converted rapidly into free $V.B_1$ when excreted, where the features of the excretion are quite similar to those of free form.

4. Biochemical Studies on phospholipids. IV. The structure of sphingosine. Kimiyoshi Ohno, p. 32–35; cf., **18**, 151, 159 (1944); **19**, 133 (1948). Sphingosine (I), N-acetylsphingosine (II), and N-benzoyl-sphingosine (III) were oxidized with Pb-$(AC)_4$, and their products were examined: for alkyl aldehyde (R.CHO), I (+), II (±), III (−); for HCN, I (+), II (−), III (−); for HCHO I (+), II (+), III (−). It has been concluded that the structure of I is octadecen (4, 5)-diol (1, 3)-amine (2).

Galctosido-sphingosine, prepd. from cerebron was also found to be oxidized with Pb $(Ac)_4$ to alkyl aldehyde (R. CHO), which gave the evidence for the linkage between galactose and C_1 of I.

5. Fractional determination of aromatic hydroxy-and amino-compounds. II. Fractional determina-

tion of IB-prositive substances. Yasuyuki Shishikura, p. 137–139. cf., **19**, 145 (1948). Phenol (I), salicylic acid (II), salicyluric acid (III), aniline (IV), and anthranilic acid (V) have been separately estimated from their mixts. by fractionations with solvents: from 5 ml. of the mixt. readily adjusted to pH 7, I is extd. with $CHCL_3$, to which 5 ml. $N/5$ NaOH is added to move I into water layer; to the residue after extn. of I, add 1 ml. of 50 g./dl. H_2SO_4 to ext. II with $CHCl_3$, which is further moved into phosphate soln; from the residual mixt. free of I and II, III is extd. with ether after addg. 3 g. of $KHSO_4$; the final residual mixt. is adjusted to pH 3 with NaOH, from which V is extd. with butanol.; for the extn. of IV, the initial mixt. is adjusted to pH 9 with borax soln., to which color reagt. is added. After standing for 10 min. at room temp., the colored soln. due to IV is extd. with xylol for colorimetry.

6. On the fate of sterols in animal body. II. Perfusion experiments of dehydrocholestein, ergosterin, dehydrocholic acid, and unsaturated bile acids. with frog-or rabbit- liver Susumu Tsuboi, p. 139–143. After perfusion of Ringer's soln. contg. 0.65% NaCl through frog liver under 250–280 mm. at *A. gastrohepatica*, and 40–60 mm. at *V. porta* or *V. coecalis* for 5 hrs., each 2 ml. of control (Ringer's soln.) olive oil (I), 0.1% 7-dehydrocolicosterorin (II), 0.1% ergosterin (III), or 3 kinds of 0.1% unsatrd. bile acids, (IV), (II, III, IV, were dissolved in I) was further administered by perfusion. Bile juice output per hour and Pettenkopfer's reaction were examined: bile juice output was markedly decreased by II, considerably by IV, compared with that by I, but somewhat increased by III; with controle case, Pettenkopfer reaction was strong initially, became negative reaction at 4th hr., but by II, III, or IV perfusion, the reaction returned to positive at 5 hrs.

Perfusion of 3 g. dehydrocholic acid (V) with rabbit liver gave the following results; from bile juice of gall bladder, cholesterol and glycodesoxycholic acid were isolate; from perfused blood, 50mg. of desoxycholic acid fraction still in oily state was obtained.

7. Syntheses of β-iso-dihydroxycholenic acid derivatives. Taëi Shimizu, Taro Kazuno, & Kenji Matsumoto, p. 144–147. Syntheses of 3,7-diacetoxy-12-kotocholanic acid, 3,7-diacetyoxy-11-(α)-brom-12-ketocholanic acid, 3,7-diacetoxy-11-(β)-brom-ketocholanic acid, 3-acetoxy-12-ketocholadienic acid, 3-oxy-12-ketocholadienic acid, 3,12-dihydroxycholadienic (iso-dihydroxycholadienic) acid, 3-acetoxy-12-ketocholenic acid, β-iso-dihydroxycholenic ($\Delta^{9,9}$-3,12-dihydroxycholenic acid), and α-apocholic acid were described starting from cholic acid.

8. Colorimetric determination of blood chloride. Kishima Imai, p. 147–149. To remove protein, add 0.05 g. of powdered borax-Zn acetate mixt. to 10 ml. of dil. blood (100 times), and filter. To 1.0 ml. of the filtrate, add 4 ml. of alcohol (95%) and ca. 0.05 g. of powdered Agchromate mixt., and filter. To 1.0ml. of the filtrate, add 3 ml. of 10% AcOH and 1 ml. of 0.2% diphenylcarbazide dissolved in the mixt. of 5ml. of 10% AcOH and of 495 ml., 95% EtOH, dilute to the 10 ml.-mark with water, let stand for 10 min., and measure with colorimeter against stand. NaCl soln. (0.0355 mg. Cl/ml.).

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1. The action of rabbit liver enzyme on quinoline derivatives. Ryoji Ito, Yukichi Hashimoto Ü Sadao Kitaura, p. 27–30. With 20% water exts. of rabbit liver, 30 quinoline derivatives, 11 quinine salts, and their related compds. were examined for the oxidation by use of Thunberg's tube or Warburg's manometer. Among these sabstrates, 3-, 4-, 6-, and 7-methyl-quinoline, acridine, 9 quinine salts, quinochonine, and optochine showed to reduce MB at pH 7.0 at approx. same rate as quinoline did; from O_2-uptake, one atom of oxygen was found to be used for the oxidation of one molecule quinoline; the reduced activities for quinine, quinoline, or acridine of 10 % exts. of rabbit liver readily washed in the slice state for 2 hrs., regained their original activities by the addn. of 10% boiled exts. of rabbit liver or 0.01ml. of satrd. FAD; as the end-products, 2-hydroxyquinoline, and 2′-hydroxy-6′-methoxy-3-vinylruban-9-ol are isolated from quinoline, and quinine, respectively.

2. Fractional determination of aromatic hydroxyl-and amino-compounds. III. The determination of IB positive substance in urine. Yasuyuki Shishikura, p. 31–33: cf., **19**, 145 (1948); **20**, 137 (1948). Prior to the fractional detns. of phenol, aniline, salicylic, salicyluric, and anthranilic acids in urine by the previously reported method, the urine was primarily treated with diatomaceous earth (Kieselguhr), and HCl, and secondarily with Pb $(OH)_2$ and NaOH. With the filtrate obtained, the fractional detrns. of phenol, aniline, solicylic, salicyluric, and anthrsnilic acids, were carried out.

3. Thə fate of sterols in animal body. III. Perfusion experiments of 7-dehydrocholesterol, and ergosterol through rabbit liver. Susumu Tsuboi, p. 34–40: cf., **20**, 139 (1948). (A) By the perfusion of each 0.1 g. of 7-desoxy-cholesterol (I) with 16 rabbit livers, there were obtained 0.7 mg. of glycodesoxycholic acid from the total bile juice excreted (44.5 ml.); 0.5 g. of desoxycholic acid (II) and 5 mg. of Harmmarsten positive—neutral substances in cholic acid fraction (III) from total perfused blood (2106 ml.); 10 mg. of II from total livers (1327.5 g.); the administered I was not found either in blood, or in liver. (B) Ergosterol perfusion expts. with 70 rabbit liver (60mg. to each) resulted the isolations of 2.1 g. of II from bile juices (133.7 ml.); 0.047 mg. of dihydroxycholanic acid (an isomer of II) with b.p. 195°, 10mg. of oily substance in III fraction, and 28mg. of oily in II fraction from blood (5888 ml.); 18 mg. of III, and 40 mg. of II from livers (4225 g.); 400 mg. of ergosterol was found unchanged in blood. (C) Control livers of 50 rabbits yielded 1,500 mg. of II from bile juice (106.3 ml.); 6 mg. of oily substances, in III-fraction, and 10 mg. of oily substance in II-fractions from blood (4312 mlf) respectively; 10.4 mg. of oily substance in III-fraction, and 24 mg. of oily substance in II fraction from livers (3467 g.). These oily substances showed negative Hammarsten and Liebermann reactions.

4. The composition of bile juice of *Ursus Matritimus* (Phipp's), and of *Ursus Thibetanus Japonicus* (Schleger). Tsuneharu Takuma, p. 74–75. From 30 ml. of bile juice of *Ursus Thibetanus Japonicls* (Schleger), 1.5 g. of ursodesoxycholic acid (I) and 0.6 g. of Ba - chenodesoxycholic acid (II) were isolated, but no cholic acid (III) was found. On the other hand, there was found 1.2 g. of III, and 0.7 g. of II, but none of I, in 30 ml. of bile juice of *Ursus Matritimus* (Phipp's).

5. The decomposition of vitamin A by various factors. I. The decomposition of vitamin A by exposure to air, heating, or ultra-violet-ray irradiation- Kaizo Ariga, p. 76–81. During the exptl. treatments of cod-liver oil (Commercial) (I) and Biosterin (Riken Co.) (II) dissolved in olive oil, vitamin A (V.A.) content and peroxide number (P.N.) have been detd. by Carr-Price's, and Lea's methods, respectively. (a) On exposure to air, the decreases of V.A. are much more rapid in II than in I, the latter having greater P.N. The features of P.N. increases are inversely corresponding to those of V.A. contents; (b) Heating ($98°\pm1°$) causes greater reduction of V.A. in II than in I the amts. of which are corresponding to heating time. P.N. is straightly increased in I, but almost const. in II; (c) Ultraviolet-ray irradiation results stronger decomposition of V.A. in II than in I as observed in (c); (d) In progress of exposure to air after heating or irradiation, the decreases of V.A. are gradual in I, but in II it is rapid initially, and becoms slower; (e) By the addn. of 0.1 ml. of 10% hydroquinone to 1 ml. of II (20,000 V.A. units), no reduction of V.A. content is observed.

6. The distribution of cholinesterase in the tissues of different animals. Genshu Wakabayashi & Masabumi Sato, p. 81–85. Fasting animals were killed by taking blood from vein. 0.2 ml. of dil. serum with Ringer soln. (1 : 3), or 0.2 ml. of the supernatant of tissue brei prepd. by grounding 2 g. of each tissue with 20ml. of Ringer soln. was used for the detn. of cholinesterase activity by Ammon's manometric method with 1.5 ml. of 0.4% acetylcholine (Roche) dissolved in Ringer soln. under 5% CO_2-95% N_2 mixt. at 37°, and evolved CO_2 (μ l.) per 0.1 ml. of serum or per 0.1 g. of each tissue after 30 min. incubation are calcd. for the comparison between serum, liver, kidney, *etc.*, of rabbit, rat, mouce, guinea pig, *etc*, dog, etc. Enzyme activity was (a) with serum, mouse$\gg$dog$>$human$\gg$cat—guinea pig$>$rat$>$rabbit; (b) with liver, dog$\gg$mouse$\gg$rabbit$>$rat$\gg$guinea pig; only in guinea pig, the activity of serum was higher than that of liver.

7. Studies on the acid-hydrolysis of polysaccharides. I. On the reducing power appeared by the acid-hydrolysis of starch. Tsunetaka Kushimoto, p. 86–89. To each 5.0 ml. of 1% potato starch soln., add 5.0 ml. of $1\,N\sim5\,N\,H_2SO_4$, $1\,N$·HCl, or $1\,N$ oxalic acid, and immerse in a boiling water-bath for 5–120 min. After the incubation, neutralize with NaOH and dilute to 50ml. with dist. water. With an aliquot, the reducing power (R.P.) was detd. by Hagedron-Jansen's method. The following results were obtained: (a) the rate of R.P. was increased with time, $1N$HCl$>$

$1N\,H_2SO_4 > 1N$ oxalic acid; (b) the rate of R.P. increase was almost proportional to the concn. of H_2SO_4; (c) varying concns. of starch (1.0–3.0%) resulted somewhat higher rate of R.P.-increase at initial period (30–40min.), proportionally to the concn. of starch.

8. Studies on the acid-hydrolysis of polysaccharides. II. On the interrelation between rotatory and reducing powers appeared by the acid-hydrolysis of starch. Tsunetaka Kushimoto, p. 119–122. cf., **21**, 86 (1949). Specific rotatory power (S.R.P.) was decreased with progress of the acid-hydrolysis, and the rate of S.R.P. reduction was nearly parallel to the concn. of acid. S.R.P. reduction was not so remarkable when glucose was incubated with 1–4 N H_2SO_4. It has been interpreted by the author that glucose appearing during the acidhydrolysis may exist in labile state.

9. The distribution of nicotinic acid in animal and vegetable tissues. Kazuo Kawashima, p. 122–129. The contents of nicotinic acid have been detd. by previously reported method (**19**, 149 (1948), with nearly 150 vegetable and 120 animal tissues and foods.

10. Quantitative determination of vitamin B_1 by use of permutite. I. On the determination of vitamin B_1 in its pure soln. and in urine. Yoshitsugu Nose, & Toshio Tashiro, p. 130–134; cf., **19**, 149 (1948). Active permutite has been prepd. as follows: sift out the commercial permutite (Takeda Co.) through a 60–80 mesh sieve, wash first with water several times, then twice with 10 ml. of 3% boiling acetic acid soln. in a water bath at 100°, subsequently with 5 vols. of 25% KCl, further twice with 3% acetic acid, and finally repeat water-washings until no turbidity occurs with the supernatant by the addn. of $AgNO_3$. Thus prepd. active permutite should kept in water for the following analytical procedure : an adsorption tube made of glass, consisting of the upper part (8 cm. hight × 2 cm. diameter, 25 ml. of volume), of the middle part (15 cm. × 0.7 cm.), and of the under capillary (1cm. × 0.5 mm.); put a small amts. of glass wool into the bottom of the middle tube, fill with water, pack the active permutite to the 2.5 ml.-mark of the middle part, and push it, so that the amts. of filtration may be 1 ml. per min.; pour the specimen soln. into an adsorption tube, wash several times with each 10 ml. of water, until no fluorescence is found in the filtrate; pour 15 ml. of 25% KCl–0.1 N HCl mixt. immediately after boiling, and all the collectep filtrate is followed by the addn. of 0.1 ml. of 1 % $K_3Fe(CN)_6$, and 3 ml. of 30% NaOH; add 8–10 ml. of butanol, shake throughly for 2 min., and centrifuge; after adding 1 g. of anhydrous Na_2SO_4, 5 ml. of the anhydrous layer was used for fluorescentometry. These conditions of the procedures, and reagts. were criticized, and applicated to the $V.B_1$ test of urine.

11. On the reaction between hemoglobin and ascorbic acid. I. The formation and the decomposition of choleglobin. Daizo Takeya, p. 134–140. The mixt. of 4 ml. of oxyhemoglobin (HbO_2) soln. (50 times diluted soln. of well washed erythrocytes) at pH 6.1, and 10ml. of 0.7% ascorbic acid was incubated at 38°. At one hour interval, the mixt. was treated with 5 ml. of glacial

acetic acid, to dissolve the ppts. formed and extinction was measured with Pulfrich-photometer by use of Filter S_{61}· E_{61} (extinction at 610 mμ) reached to the max. value at 6th hr., and turned to lower nearly to the original value at 17th hr. When catalase prepd. from the same HbO_2 soln. by CO_2-aeration and then by MeOH pptn. was added to the reaction system, the formation of a substance responsible to the absorption at 610 mμ (610-subst.) was delayed at first 3 hrs. then rapidly increased, and finally reached to the max. value of the control at 6–7 th hr. The addn. of heat-treated (52° or 60°) catalase showed nearly similar curves of the 610-subst. formation.

By the addn. of NaOH to the reaction mixt. during the incubation, red-color developed, which was, however, not positively detd. if red color was due to pentdyopent compd. or merely to ascorbic acid.

The amts. of Fe liberated from the reaction mixt. with 1.2% HCl, at 380 for 5 hr. was detd. by colorimetry using. *o*-phenauthioline: Fe was increased almost proportionaly to time, and reached to the max. (0.12 mg.) at 4th–5th hr., while Fe in the control was nearly const.

ABSTRACTS

from

The Journal of Japanese Biochemical Society (Seikagaku)
Volume 22 (1950) p. I—VIII.

1. On the metabolism of the bacteria isolated from decayed teeth. Toshizo Kurokochi, p. 1—6. The following bacteria were isolated from decayed teeth: *Lact. acidophilus* (I) and *Staph. citreus* (II) from the surface part; *Lact. odontolycus* II Rodolinque (III) and *Staph. albus* (IV) from the inner part. Each of them was cultivated in 40 ml. of 1% glucose-broth, washed 3 times with saline soln., and suspended in 20 ml. of saline soln. for metabolic expts. By use of Thunberg's tube contg. 1 ml. of each substrate (*M*/40—*M*/160), 1 ml. of the bacterial suspensin and 1 ml. of methylene blue (1 : 5000), the following results have been obtained: (a) Hexose is generally metabolized by I and II. The order of the utilization of substrates by II are fructose, galactose, mannose > glucose > maltose > sucrose > lactose; (b) Among fatty acids, formic and butyric acids are metabolized by I, and II, resp.; (c) The metabolism of dicarboxylic acids are seen remarkably by II as follows: malate > succinate >malonate; (d) Amino acids: glutamic acid, leucine and glycine are metabolized by both I and II; (e) Glycerin is metabolized remarkably by III, slightly by I and II; IV scarcely metabolizes the substrates. To each bacteria cultivated in 10 ml. of 1% sugar-broth at pH 6.5, 37° for 24 hrs., 90 ml. of the same broth was added and an aliquot was examined for detns. of redox-potential (E_h) pH, and acid formation at intervals of 3 hrs. (a) acid formation: maltose>sucrose>starch (of both wheat and rice)> glucose>potato starch>control>lactose; (b) pH varies approx. in parallel with acid formation; (c) E_h was lowered to the min. value (−200 ~ −300 mv.) at 6th hr. Each bacterium was cultivated in the medium contg. Na-citrate (40 g./*l.*) $MgSO_4$ (1.0 g./*l.*), NH_4Cl (2.5 g./*l.*), and on the addn. of substrates, the total gas, CO_2, CH_4, H_2, and N were measured: CH_4, or CO_2 was poorly formed; amino acid yielded a trace of N_2.

2. On the oxidized form of vitamin B_1. Shunji Mizuhara, p. 7—11. Vitamin B_1 (V. B_1) and co-carboxylase are oxidized at pH 7.4 by aeration of O_2, which can be inhibited by the addn. of cysteine. Neithe -S-S- nor -SH are detectable in the oxidized V.B_1 by polarography. The end-product of the oxidized V.B_1 by O_2-aeration is identified as 2-methyl-4.5-diaminomethylpyridine.

3. The fate of vitamins B_1 and B_2 *in vivo*. Shunji Mizuhara, p. 11—15. The perfusion of 3 times dild. blood with Ringer's soln. at pH 7.4 contg. dehydrochlolic acid (I), vitamins B_1 (V.B_1), or B_2 (V.B_2) with rabbit liver have given the following results of their blood contents, liver deposits,and biliary excretion: (a) The perfusion of I (100 mg.)—(i) desoxycholic acid: 5 mg. in

blood, 18 mg. in liver, 311 mg. in bile juice; (ii) cholic acid (II): 15 mg. in liver, 11 mg. in bile juice; (b) The perfusion of I (100 mg.) and $V.B_1$ (1 mg.)—$V.B_1$: 297 γ in blood, 469 γ in liver and 52.5 γ in bile juice (recovery, 819 γ): (c) The perfusion of I (100 mg.), $V.B_1$ (1 mg.) and $V.B_2$ (1.5 mg.)—(i) $V.B_1$: 221 γ in blood, 702 γ in liver, 67.9 γ in bile juice. (recovery, 998 γ); (ii) $V.B_2$: 455 γ in blood, 264 γ in liver, and 753 γ in bile juice. (recovery, 1,472 γ); (d) The perfusion of $V.B_1$ (1 mg.), $V.B_2$ (1.5 mg.) and II (100 mg.)—(i) $V.B_1$: 308 γ in blood, 658 γ in liver, 21.7 γ in bile juice. (recovery, 988 γ); (ii) $V.B_2$: 615 γ in blood, 644 γ in liver, 229 γ in bile juice. (recovery, 1,488 γ).

4. On the influences vitamin B_1 and B_2 upon the excretion of bile juice and upon the metabolism of bile acids in rabbits. Tatsumi Ogawa, p. 15—21. By perfusion expts. with rabbit liver, the excreted bile juices have been examined for the influences of vitamins B_1 ($V.B_1$) and B_2 ($V.B_2$) or cholic (I) and dehydrocholic acids (II). (a) The excretion of bile juice: control, 2.02 ml.; $V.B_1$ (1 mg.) addn., 3.02 ml.; $V.B_1$ (1 mg.), $V.B_2$ (1.5 mg.), and II (0.1 g.) addns., 9.01 ml.; (b) Bile acids: from the control, 30 mg. of desoxycholic acid (III) and 52 mg. of glycodesoxycholic acid (IV) are obtained, while in $V.B_1$ (1 mg.) addn. group 38.3 mg. of the coupled compd. of I, 78 mg. of IV, and a trace III are found; when $V.B_1$, $V.B_2$ and I (0.1 g.) are perfused, 16 mg. of III is found only in liver, 12 mg. of reductodehydrocholic acid (V) is obtained from blood, and 257 mg. and 12 mg. of I are recovered in bile juice and blood, resp.; by perfusing $V.B_1$, $V.B_2$, and II (0.1 g.), 98 and 16 mg. of III are found in bile juice and liver, resp., while only 14 mg. of II is recovered from blood; 120 mg. of 7,12-diketocholanic acid, and 9 mg. of V are further obtained from bile juice. (c) Vitamin C addn. to perfusing liquid increases the coupled compds. of III, and rather decreases the free form of III.

5. On the estimation of vitamin B_1 by use of permutite. II. Yoshizugu Nose and Toshio Tashiro, p. 21—25; *cf.*, **21** 130 (1949). The contents of vitamin B_1 ($V.B_1$) in blood have been detd. as follows: mix 5 ml. of blood with 25.0 ml. of water and 0.5 ml. of 1*N* HCl; to 10 ml. of the mixt., add 2.0 ml. of 0.6% Taka-diastase soln. previously treated with diatomaceous earth (Kieselguhr); incubate at 40—45° for 1—1.5 hrs.; add 0.3 ml. of 1*N* HCl, immerse in a water bath at 80° for 15 min. by stirring; add 5.0 ml. of 10% metaphosphoric acid soln., and centrifuge at 3000 r.p.m. for 15 min.; to the supernatant add 1*N* NaOH to pH 4.5, pour into a permutite-pile, passing through it at the rate of 1 ml./min., and elute with 15 ml. of boiling KCl-HCl mixt.; to the eluate add 1.1 ml. of 0.1% ferricyanate and 3 ml. of 30% NaOH. This method has been thus modified with reference to the rapid hydrolysis by use of metaphosphoric acid, and to the extn. by warming after the hydrolysis.

6. Ibid. III. Yoshizugu Nose, Toshio Tashiro, and Shunichi Kozuka, p. 25—28. The modified dermutite method has been appied to the detn. of vitamin B_1 contents in animal and vegetable tissues.

7. On the cholinesterase of dog liver. Akira Tamai, p. 29—32. Cholinesterase (I) has been purified as follows: (a) grind dog liver, ext. with 2 vols. of water for 4 hrs., and centrifuge; to the supernatant add $(NH_4)_2SO_4$ (final concn., 20 g./dl.), weakly acidify with $N/2$ AcOH, and 1 hr. later centrifuge; from the obtained supernatant, ppt. the enzyme with $(NH_4)_2SO_4$ at 0.4 satn. in weak acid reaction with AcOH, and centrifuge after standing for 1 hr.; to the ppts., add $(NH_4)_2SO_4$ (35 g./dl.), and centrifuge after standing overnight; dissolve the ppts. in a small amt. of water, add $(NH_4)_2SO_4$ to a final concn. of 25 g./dl., let stand for 1 hr., and centrifuge; from the obtained supernatant ppt. the enzyme with $(NH_4)_2SO_4$ at 0.4 satn. in weak acid reaction and centrifuge; dissolve the ppts. again in a small amt. of water, and dialyze against running water; discard the formed ppts. by centrifugation, and keep the supernatant (Prepan. A). (b) To the water exts. of dog liver add $(NH_4)_2SO_4$ (final concn., 25 g./dl.), let stann for 1 hr., and centrifuge; from the obtained supernatant, ppt. the enzyme with $(NH_4)_2SO_4$ at 0.4 satn. in weak acid reaction with $N/2$ AcOH and centrifuge after 1 hr.; dialyze the ppts. and separate the supernatant by centfirugation. (Prepn. B) By manometric method, the activities of Prepns. A and B for acetylcholine are found to 135, and 118 μl. of CO_2 evolution per mg. (dry wt.) for 30 min. The activity of I will be kept as long as 10 months at 2—4°. The optimal concn. of acetylcholine, 0.025 M; the optimal pH 7.4—7.6; the activity of I is strongly inhibited by the addn. of acetone, and completely lost by 50 vol.%.

8. On the difference in quality between erythrocytes and serum cholinesterase. Akira Tamai, p. 32—37. Cholinesterase (I) activity has been detd. manometrically according to Ammon's method. (a) The opt. concn. of the acetylcholine (II) as substrate is 0.0025 M; with erythrocytes, while in case of serum, the activity of I is increased almost proportionally to the concn. of II as much as to 0.025 M; (b) Opt. pH: erythrocytes, pH 7.2—7.4; serum, pH 7.4—7.6; (c) Eserin inhibition: serum> erythrocytes. These findings are not the case with I of rabbit blood.

9. A note to the determination of blood cholinesterase actitity. Akira Tamai, p. 37—41. The auther has suggested to represent the activity of erythrocytes cholinesterase (I) by I-index and I-quotient, which are represented by the ratio of CO_2 evolved (μl.) per ml. of blood for 30 min. to erythrocytes counts per μl. of blood, and to hematocrit value, resp. The activity of serum cholinesterase (II) can be also represented by [CO_2 evolved (μl.) for 30 min. by 0.1 ml. of serum]×([serum volume]/[whole blood volume]). These ratios have been calcd. out with many specimens of human blood from the detns. of CO_2 evolution, hematocrit value, and erythrocytes counts.

10. On the influence of methionine upon the metabolism of phosphorus in rat liver. Shozo Tanaka, p. 41—45. The rats were fed for 6 days with daily 10 g. (dry wt.) of the low protein diet of the following compositions casein (5%), soy bean oil (15%), starch (70%), McCollum's salt mixt. (5%), Ebios

(5%), cod liver oil (I drop). After fasting for 24 hrs., 1.5 ml. of $Na_2HP^{32}O_4$ (1.66 mg. P/ml.) was administered by injection. The rat was sacrificed, and liver was fractionated into lipidic-P (I), mineral-P (II), acid-sol. organic P (III), and acid-insol. P (IV). P was detd. by Allen's method, and radioactivity of P^{32} was estd. after dry digestion. (a) The turn-over of P in liver, cald. from [P^{32} administered]/[P^{32} found] was greater in the following order: II>III >I>IV; (b) The injection of 0.1 g. of methionine (V) increased the turnover of I, and to a smaller extent, of IV; (c) The injection of 0.4 ml. of brombenzene (VI), decreased the turn-over of II, III, and I, but rather increased that of IV; (d) by the injection of 2.0 ml. of 0.5% CH_2ICOOH (VII), the turn-over of P was decreased, remarkably in IV-fraction; (e) Simultaneous administration of V and VI inhibited the action of VI alone; (f) V and VII administration was also antagonistic against the lowering action of VII alone.

11. Studies on aneurinase. VI. On the properties of shellfish-and feces-aneurinase. Kaoru Kaminishi, p. 45—53; *cf.*, **18**, 63, 325, 339 (1944). With shellfish (*Corbicula atrata*) (I)- and feces (II)-aneurinase the following properties have been observed: (a) Opt. pH: I, pH 5.5; II, pH 6.5 (b) Opt. temp.: I, 55°; II, 38°; (c) I is rather thermostable (inactivated at 90° for 20 min., or at 100° for 10 min.); II is thermolabile (inactivated at 60° for 30 min. or at 80° for 5 min.); (d) II does not act on vitamin B_1 when gassed with H_2, while I acts both aerobically and anaerobically; (e) SH-compds. such as glutathione, cysteine. NaSH, $Na_2S_3O_3$, *etc.* promote the activity of I, but give no influence on that of II; (f) Fe^{++} promotes I, and Fe^{+++} rather inhibits II.; (g) Pptn. with acetone strongly reduces the activity of I, but not so markably that of II (h) By dialysis only I is fractionated into apo- and co-enzymes, which can be resynthesizable but this is not the case with II; (i) Opt. redox-potential: I, $+31\sim41$ mv. at pH 5.5 at both aerobically and anaerobically; II, $-62\sim-103$ mv. at pH 6.5 at 38°, only aerobically.

12. On the distribution of copper-colloid in animal body. Shozo Tanaka, p. 54—56. The labeled Cu^{64} was ground with glucose and the mixt. was dissolved in boiling water. After filtration 0.037% Cu-colloid soln. (I) was obtained. To rabbit each 10.0 ml. of I was twice intraven. injected at an interval of 1 hr. About 6 hrs. later, the rabbit was killed, and the radioactivity of C^{64} in various organs was detd. (a) The ratio of [Cu^{64} found]/[Cu^{64} administered] was as follows: liver, 9.9—13.3%; lung 1.7—2.3%; kidney, 0.10—0.11%; spleen, 0.2—0.4%; adrenal, 0.003—0.007%. With liver of a rabbits killed about 14 hrs. after the administration, the significant increase of Cu^{64} was observed; (b) The treatment of liver with 20% CCl_3COOH, or with 5% HPO_3 can not liberate the whole Cu, 10--20% being still unliberated; (c) 12 hrs. before the administration of Cu^{64}, mouce was injected with 0.5 ml. of 1.0% trypain-blue, 0.5 ml. of electrargol, 0.1 ml. of aequozone, or 0.5 ml. of 1% iron hydroxde colloid. The amts. of Cu^{64} in liver and lung were increased strongly by iron colloid, slightly by aequozone, but not by others.

13. Studies on the acid-hydrolysis of polysaccharides. III. On the regularity and the irregularity in the process of the acid-hydrolysis of starch. Tsunetaka Kushimoto, p. 57—59; *cf.*, **21**, 86, 119 (1949). During the hydrolysis of starch (20 g./dl.) at 100° with 1*N* oxalic acid or 1—4*N* H_2SO_4, an aliquot was pipetted out from time to time for the detns. of reducing powers, and the max. I_2-starch color reaction. The author has suggested that in case of the regular hydrolysis the reducing power should be 50% as much as that on the complete hydrolysis, at the time when no I_2-reaction occurs, and such a regular hydrolysis can proceed with each acid in a certain concn.

14. The microestimation of urinanary sulface. Nobuo Tamiya, p. 59—62. (a) Inorganic S detn.: with 10 ml. of urine, prepare phosphate-free filtrate by Fiske's method (J. Biol. Chem., **47**, 56 (1921)); to the filtrate add 1 ml. of 10% AcOH and 2 ml. of 0.01 *M* Ba-acetate; let stand for 30 min., and centrifuge; in 5 ml. of the filtrate dissolve a small amt. of solid Na-acetate, add 0.7 ml. of *M*/25 $K_2Cr_2O_7$; mix. and cool well with ice for 15 min.; ppt. the formed Ba-chromate, wash the ppts. twice with each 8 ml. of Na-acetate soln. (1 : 2); dissolve the ppts. in 2 ml. of 2*N* HCl, and the mixt. is titrated with 0.012 *M* $FeSO_4 \cdot NH_4(SO_4)_2 \cdot 6H_2O$ by use of an indicator (0.04% Ba-dephenylsulfamine-sulfonate); (b) Total S detn.: hydrolyze the phosphate-free filtrate with 6*N* HCl in a wather bath for 2 hrs.; neutralize the hydrolysate with 10% NaOH; acidify by addg. 5 ml. of 10% AcOH, dilute to 50 ml. with water, and filter; to 5 ml. of the filtrate add 2 ml. of 0.07*M* Ba-acetate, and centrifuge after standing for 30 min.; with 5 ml. of the filtrate corry out the above described procedures.

15. Studies on the acid-hydrolysis of polysaccharides. IV. Starch and its intermediary hydrolysates. The structure and the polymeric degree of starch. Tsunetaka Kushimoto. During the hydrolysis of starch soln. with 2*N* H_2SO_4 at 100°, an aliquot was pipetted out for the reaction with *N*/100 I_2, the color intensity of which was estd. by use of Reitz-Duboseq's colorimeter with red filter. On the other hand, a series of starch-erythrodextrin mixt. was prepd., and its I_2-color reaction wrs also photometrically estd. Achrodextrin was also detd. as undialyzable substance. From kinetical interpretation of the exptl. results of starch hydrolysis, the following conclusions have been suggested: (a) The hydrolysis of starch is a series of cotinuous reactions, which consists of a main and two accessory reactions; (b) The main reaction starts from micro-starch molecule; (c) One of accessory reaction is the cleavage of side-chains; (d) Erythro- and achro-dextrin are rich in variety, which is probably due to the side-chains of micro-starch molecule; (e) Micro-starch molecule is calcd. to consist of 15 maltose molecules or 30 glucose units.

16. Vitamin C-like substance formed by the hydrolysis of fructose with hydrochloric acid and its inhibitory action on the autoxidation of vitamin C. Eiichi Yamaguchi, p. 71—76. During the hydrolysis of 5% fructose soln. with 3 vols. of 2*N* HCl

at 100°. an aliquot was pipetted out from time to time, and its reducing power was detd. by use of 2,6-dichlorphenol-indophenol (I). The increase of I was very rapid initially and turned stationary at 120th min. The turbidity was formed at initial period, but disappeared at 180th min., while the ppts. was greatly formed. The intensity of red-brown color in HCl soln. was max. at 120th min., while that of yellow color on the addn. of 2% metaphoric acid after neutralization was highest at 60th min., faided at 180th min. Seliwanoff's reaction was strongest at 70 min., and turned negative at 180th min. Each aliquot was mixed with vitamin C (V.C) and heated for 10—60 min', and the reducing power was estd. with I. The hydrolysate pospossessing highest reducing power and strongest Seliwanoff's reaction demonstrated the highest recovery of added V.C. The hydrolysis of oxymethlfurfurol yielded the reducing power at earlier period, and gave stonger recovery of V.C. than that of fructose.

17. The decomposition of vitamin A by various factors. (Resume) Kaizo Ariga, p. 77; *cf.*, **16**, 161 (1941).

18. Ibid. II. On the influence of salts upon the decomposition of vitamin A. Kaizo Ariga, p. 78—82. *cf.*, **21**, 76 (1949). Unsaponified substance of cod liver oil (I) was extd. with ether. Ether exts. (II) and I have been examined for vitamin A (V.A.) contents and the amts. of peroxide (P.N.) with of without the addn. of inorganic salts. (a) The amts. of V.A. is decreased in parallel with the increase of P.N., but not influence by KI, K_2CO_3 but $CaCO_3$ shows rather promotion of V.A. decompn. (c) When KI is added directly to I, the former I_2 decomps. V.A., but the addn. of KI to II inhibits V.A. decompn. by I^- ion; V.A. decompn. is promoted by acid salts, such as $Al_2(SO_4)_3$, $MgSO_4$, and $(NH_4)_2SO_4$, somewhat by $CuSO_4$ and $CaCl_2$. (e) Neutral salts: Na_2SO_4 moderately promotes V.A. decompn. (f) Basic salts: K_2CO_4, and Na_2CO_3 defend V.A., decompn.

19. Studies on aneurinase. VII. The quantitative determination of vitamin B_1 by use of aneurinase. Kaoru Kaminishi, p. 89—91. *cf.*, **18**, 63, 325, 339 (1944). Aneurinase has been prepd. as follows: grind 2 g. of the visceral parts of *Cyterea Meretri* with 6 ml. of water, adjust to pH 4.5—5.0 with 1*N* HCl, immerse in water bath at 30° for 15 min., and centrifuge. The supernatant contains anenurinase soln. (I), decomposing 2.0 γ of vitamin B_1 ($V.B_1$) at pH 5.5 at 55° for 1 hr. per ml.; incubate 10 ml. of twice dild. I with 1.0 ml. of cocarboxylase (II) (1 γ) or 1.0 ml. of $V.B_1$ (1 γ), and 2.0 ml. of citrate buffer at pH 5.5, at 100° for 10 min., adjust to pH 4.5 with 1*N* HCl; and Take-diastase soln., heat at 45° for 1 hr., treat with permutite, add K-ferricyanate, ext. with butanol, and titrate. The decompn. of $V.B_1$ and II are calcd. to be 96 and 80%, resp.; the recovaries of $V.B_1$ by the enzymatic method are 95 and 94%, with urine and yolk, resp., as much as those by thiochrome method.

20. Quantitative determinations of arginine and glycocyamine in urine, and glycocyamine-index to represent liver function. Hiroyoshi

Moteki, p. 92—96. (a) Adsorption of arginine (I): pour 5 ml. of dild. urine (5—10 times) into a tube packed with permutite (diameter, 0.1—0.3 mm^3; length, 10 cm.), washed out the remaining glycocyamine (II) by addg. 0.3% NaCl and collect the filtrate: (b) Detn. of II: dilute the filtrate to 10.0 ml. with water, cool at 0° for 5 min., add 1.25 ml. of the mixt. of 20% urea and 0.2% α-naphthol (1.5 : 0.2), let stand for 10 min., add 0.75 ml. of Na-hypobromite soln. (0.66 ml. of Br_2 dissolved 100 ml. of 5% NaOH), and 30 min., later, compare the developed color by Pulfurich's photometer with S_{53}: (c) Detn. of I: add 0.3% NaCl to 5.0 ml. of dild. urine to the 10.0 ml.-mark, and carry out the procedures as described in (b), to obtain the sum of I and II. Normal value: I, 22—118 mg.; II, 21—67 mg. in 24 hrs. urine. Glycocyamine-index is defined as ([II]/[creatinine])×100: the healthy, less than 10; liver diseases, 10—40.

21. On the fatty acids formed by the decomposition of yolk by snake poison. Nobuhiko Komatsu, p. 96—102. After the incubation of 910 g. of yolk, 450 ml. of saline soln., and 160 mg. of dried snake poison for 40 hrs at 37°, the following substances have been isolated: (a) satrd. fatty acid (I)—palmitic and stearic acids; (b) unsatrd. fatty acid (II)—oleic and linoleic acid; unidentified acid with a formula of $C_{22}H_{42}O_2$. These products possess the following activities: (a) hemolysis: I> II; (b) the inactivation of diphtheria- and tetanus-toxins: I>II.

22. The action mechanism of vitamin B_1. Shunji Mizuhara, p. 102—166. The autoxidation of furol to furil in alkaline soln. is shown to accompany H_2O_2-formation, upon which the following compds. relating to vitamin B_1 ($V.B_1$) give the influence: the catalyzing action is caused strongly by cocarboxylase, moderataly by $V.B_1$, and 4ry thiazole; 3ry thiazole is rather inhibitory in its high concn; the oxidized forms of $V.B_1$ such as disulfide, S-$V.B_1$, and benzylthiazolon are not effective. On catalytic reaction by $V.B_1$, no H.O. is detectable, and most of $V.B_1$ is decomposed. $V.B_1$ also catalyzes the oxidation of arsenite, where the decompn. of $V.B_1$ is less than that in case of $V.B_1$ oxidation in alkaline soln.

23. A new fluorescent reaction of ketobile acids with ketone group. (Shimizu-Mizuhara's reaction). Shunji Mizuhara, p. 106—107. Transfer a small amts. of bile juice contg. in a test tube, add 3—5 ml. of 15% NaOH, dissolve by boiling, add 5—6 drops of 1% ferricyanate, and ext. with 2—3 ml. of butanol for fluorometry. A violett-blue and a faint yellow-red fluorescences are observed by bile acids possessing ketone groups at C_3, C_7, and at C_3, C_6, resp.

24. A modified method for the colorimetric determination of pyruvic acid. Taiji Shimizu, p. 108—114. The following two modified methods with high specificity to pyruvic acid (I) have been reported. (a) Add 2 ml. of blood to 10% CCl_3COOH, centrifuge; transfer the deproteinized supernatant into a centrifuge-tube, warm to 25° in a water bath; add 0.7 ml. of 0.5% 2,4-dinitrophenylhydrazine-2*N* HCl (DNP-reag.), keep at 25° for 5 min.; add 8 ml. of

xylene, aerate through a capillary for 3 min., centrifuge; remove water layer with a capillary pipeette, add 6 ml. of 10% Na_2CO_3 to xylene layer, aerate for 3 min., centrifuge; pipette out exactly 5.0 ml., to which 2 ml. of 4*N* NaOH is added; 5 min. later, the developed red color is detd. by a Pulrich's photomer with S_{47}. (b) Following to the above procedures of deproteinization, and DNP-reag. addn., add 8 ml. of xylene, aerate, pipette out water layer; wash xylene layer several times with each 3 ml. of water; add 3 ml. of water, shake well, adjust water layer to pH 2—3, let stand for 18—24 hrs. in an ice-chest; remove water layer, add 7 ml. of 10% Na_2CO_3, and carry out photometrical detn. with S_{47}. For the detn. of I in urine, add 0.5 ml. of 20*N* H_2SO_4 to 100 ml. of urine; to 10 ml. of the acidified urine add 0.75 g. of acid cray, for 2 min., and filter; to the filtrate add 5 vols. of 10% CCl_3COOH; with 8 ml. of the mixt. the procedure of (a) or (b) is carried out. α-Ketoglutaric, acetoacetic, and oxaloacetic acids, as well as acetone are all not interfering, having the least extinction coefficients. Procedure (b) has been found to be more specific to I than procedure (a).

25. On the calorimetric calculations of foods. Yoshiaki Miura, p. 115—119. To 3 healthy men the usual foods of Japanese were given. The administered foods and the excreted feces and urines were analyzed for N by Kijeldahl's method, fats by extg. with ether in a Soxhlet flask, and sugars by Bertrand's method. The consumptions of calories were calcd. from respiratory quotients and from time studies of every work. Calorimetric calculations were found to be most agreeable with the calcd. values from C-N balances, by using Kodama's heat values; protein, 4.0 Cal.; fats, 65 Cal.; sugar, 4.1 Cal.

ABSTRACTS

from

The Journal of Japanese Biochemical Society (Seikagaku)
Volume 22 (1950) p. IX–XV.

26. On the bile constituents of the typhoid bucilli carriers. Kazumi Yamasaki. p. 119–124. Bile juices were collected from the typhoid bacilli-carriers (I) and the non-carriers (II) by Meltzer-Lyon's method for the analyses of (a) Mucin: I, 0.76%; II, 0.27%; (b) Fatty acid fractionated by Wieland-Seibert-Heki's method: I, 0.20%; II, 0.97%; (c) Cholesterol: I, 0.07%; II, 0.11%; (d) Bile acids. (i) total bile acids: I, 0.62%; II, 1.08%; (ii) cholic acid (III): I, 0.29%, II, 0.42%; (iii) desoxycholic acid (IV): I, 0.33%, II, 0.66%. Anthropodesoxycholic acid was found to be the main compoent of IV.

27. The colliod titration method and its application to bacteria. Hiroshi Terayama and Seiji Arakwa. p. 125-128. A mixt. of 1 ml. of bacterial suspension (2 mg./ml.) and 5 ml. of 0.02% macramine (I) is titrated at pH 1∼12 with 0.000666 *N* K-polyvinylsulfate by use of toluidine blue as an indicator. All the bacteria tested were combined as "neg. colloid" with "pos. colloid" of I; Gram-neg. bacteria (II) combined much more I than Gram-pos. bacteria (III); the combined amt. of I was generally increased at higher pH which was more remarkable with II than with III; III combined I at lower pH, but this is not the case with II.

28. Studies on the acid-hydrolysis of polysaccharides. V. On the difference between acid-hydrolysis and enzymatic hydrolysis of starch. Tsunetaka Kushimoto and Kenji Marumoto. p. 128–130; *cf.*, **21** 86, 119 (1949); **22**, 57, 62 (1950). During the hydrolysis of 15 ml. of 2% starch either with 15 ml. of 2 *N* H_2SO_4 at 100° or with 0.25 ml. of saliva at 38°, each 2 ml. of the reaction mixt. was pipetted out at intervals of 1 min., to which 10.5 ml. of 50 g./dl. Na-acetate, 9.0 ml. of dist. water and 0.5 ml. of 0.1 *N* I_2 were added. The mixt. was fractionated by centrifugation into the supernatant contg. I_2-erythrodextrin (I) and the ppts. of I_2-starch (II). For the detn. of II, the ppts. were washed with 5 ml. of 50 g./dl. Na-acetate, centrifuged, dissolved in 5 ml. of dist. water by heating, and 0.5 ml. of 0.02 *N* I_2 was added. With this soln. and the supernatant of I, the colorimetry was carried out to est. II and I, resp., by use of a red filter, comparing with I_2-color of I pipetted out at 5th min., as well as with that of the original starch soln. (a) Decrease of II: ptyalin (III)> H_2SO_4; by III, starch was decreased logarithmically; but rather lineralry by H_2SO_4 (b) The appearance of I: III (max. at 4th min.)>H_2OS_4 (max. at 6th and 8th min.). These results have been interpreted that III may convert macro-starch molecule to micro-starch molecule, and the special feature of I would be due to side-chains of erythrodextrin molecule.

29. A study on the properties of iodine-starch system. Tsunetaka Ku-

shimoto. p. 131–141. The mixt. of 50 ml. of 1% sol. starch soln. and either various amts. of KI-I_2 or I_2-alcohol soln. was dild. to 200 ml. with dist. water, ranging 0.0017–0.0175 g. of I_2 per 100 ml. and the following expts. have been carried out. (a) Dilution with water: much more dilution is required for the color disappearance of starch with higher concn. of I_2; (b) dilution and heating: the temp. for thermal dissociation of I_2-starch system lowers inversely to the final concn. of I_2; with the same concn. of I_2, the temp. is KI-I_2>I_2-alcohol; (c) the intensity of I_2-starch color is increased exponentially with I_2 concn., and the max. value is found by 0.0175 g./dl. of I_2 with 0.25 g./dl. of starch.

30. On the magnetic properties of catalase. Shozo Tanaka. p. 141–143. With cryst. beef liver catalase (I) and its compds. the magnetic suceptibility ($\chi_{g.\,atom\,(Fe)} \times 10^{-6}$e.g.s.u.) has been detd. at pH 6.8 (M/450 phosphate buffer). χ of I contg. 7.24 and $9.21 \times 10^{-5} M$ Fe is reduced from 15300 and 14450 to 6500 and 6350, resp., by the addn. of $10^{-3.7} M$ KCN, which can not be appreciably changed by the successive addn. of $10^{-3.7}$ M NaN_3. The direct addn. of $10^{-3} \sim 10^{-2} M$ NaN_3 or p-cresol to I causes no significant change of χ.

31. Vitamin B_2 contents of frog eyeballs. I. Kunio Yagi, p. 143–147. Eyeballs obtained from the frog kept in a dark for 24 hrs. were analyzed for vitamin B_2 ($V.B_2$) contents by lumiflavin method. Distribution of $V.B_2$: chorioidea>[iris+ciliary muscle]$\gg$sclera>cornea>retinal; practically null in lens, aq. humor in ant. chamber, and vitreus. Eyeballs of the hibernant frogs shows no significant feature of $V.B_2$ contents, except for slightly less $V.B_2$ contents in chorioidea. $V.B_2$ of the eyeballs after exposure to light for 1 hr. is 3 times as much as that of the non-exposed.

32. The influence of nitrate upon the respiration of *Escherichia coli*. Edahiko Murakami, p. 147–152. The cells of *E. coli* cultivated in pepton-broth-agar contg. 1% KNO_3 were aerated for 8 hrs., dialyzed against dist. water for 2 days, and suspended in a mixt. of physiological saline soln.-phosphate buffer at pH 7.4. The addn. of M/300 KNO_3 accelerates the balnk O_2 uptake of the bacterial suspension (I), decreased O_2 uptake of I with succinate (II), lactate (III), or formate (IV); but rather increased that with glucose (V), acetate (VI), or pyruvate (VII). The formation of NO_2^- during the oxidation of substrates by I is in the following order: V, VI, VII> II, III,> IV. H_2-formation by I with V is markedly depressed by the addn. of KNO_3 from 250 to 84 μl. The rate of methylene blue reduction is as follows: IV, V$\gg$oxaloacetate$\gg$II> DL-alanine> citrate; but there is no significant difference in the amts. of formed NO_2^- between substrates. The respiration of I is inhibited by M/45 NaF, M/450 As^{+++}, or M/4500 monoiodoacetate. The addn. of M/450 KNO_3 readily overcomes the inhibition by As^{+++} or by NaF, but gives no effect on the monoiodoacetate inhibition.

33. On the promoting action of p-aminophenol on the decomposition of starch by iron salt-hydrogen peroxide system. Shozo Tanaka. p. 152-161. For the detn. of starch decompn.

by Fe-H_2O_2 system, the time required for the disappearance of I_2-starch reaction is examd. (a) The most rapid decompn. of starch (I) is gained by the following-system: 0.5 ml. of *M*/100 $FeSO_4$; 2.0 ml. of 1.0 *M* H_2O_2; 0.5 ml. of 1% I; 1.5 ml. of *M*/5 acetate buffer at pH 3.6. Fe^{+++} shows far weaker decompn. than Fe^{++}. The decompn. of I by Fe^{++}-H_2O_2 system is accelerated by the following substances: mono- and di-hydroxy compds. of benzene and of naphthalene; tyrosine, cysteine, tryptophan, and histidine; 6- and 8-hydroxyquinoline; but the inhibitory action is found by En-HCl, *o*- and *p*-toluidine (II), some amino acids, pyridine, 6- and 8-methylquinoline. (b) Redox-potential (E_h) rises abruptly on addg. H_2O_2 to the mixt. of *M*/100 Fe^{++} and *M*/80 either promoting or inhibiting substance at pH 3.6, where the min. E_h-difference is found between Fe^{++}-H_2O_2 system in the absence and the presence of *p*-aminophenol (III), hydroquinone, or hydroxylamine, but greater E_h-difference is observed by using II. E_h-difference is minimal on addg. H_2O_2 at the concn. resulting the max. decompn. of I in the presence of Fe^{++}. (c) On addg. H_2O_2 in acetate buffer at pH 3.6, magnetic susceptibility ($\chi_{g.\ atom\ (Fe)}$) is decreased to χ of Fe^{++} at pH 3.6; χ of Fe^{++}-H_2O_2 (at pH 3.6) is increased by III, but rather decreased by II. The promoting action of III on the decompn. of I by Fe^{++}-H_2O_2 system is attributed to the formation of Fe^{++}-III complex possessing more odd electrons, which may prevent the lowering of χ on the addn. of H_2O_2.

34. On the quantitative determination of porphyrin body in plant tissues. I. Takashi Kawaii, p. 187–189. For the detn. of free- (I) and metal combined-porphyrin (II), an improved method has been devised in respects to the use of 30 g./dl. KOH for the extn. of I and II from tissues, and the removlal of chlorophyl and its derivatives interfering the fluorescentmentry of I and II by repeated treatments with 5 g./dl. HCl. I and II thus treated can be extd. with AcOH-ether and formic acidether, resp.

35. The activity of plant inulase. Shinichi Shibuya & Kazuyoshi Tsukamoto, p. 189–191. Inulase (I) was extd. from ground plant tissues with 5 vols. of *M*/50 acetate buffer at pH 3.8. The activity of I was detd. in the following reaction mixt.; I, 5ml.; 1% inulin, 5ml.; *M*/10 acetate buffer at pH 3.8, 2.5 ml. After incubating the mix. for 24 and 48 hrs. at 40°, the reducing power was estd. by Bert rand's method. With 15 species of plant, the activity of I was approx. proportinal to the contents of fructan, but not to that of free fructose (cf., Shibuya & Nishimoto, *J. Jap. chem. Soc.*, **69**, 42 (1948)). These facts are suggesting that inulin may not be directly hydrolyzed to fructose by I.

36. Sutdies on the lipids in microorganisms. I. The influence of the cultivation temperature upon the lipids constitutents of *Penicillium chrysogenum*. Yo Imai, p. 192–195. *Pen. chrysogenum* was cultivated in the acid-Czapeck-Dox medium (pH 4.6) at 4°, 16°, 22°, or 26°. The decrease of glucose contd. in the medium was greater in culture medium at higher temp. than at lower temp. The cells were collected, washed with water, dried, and extd. with MeOH-ether (3:1) for the following

analyses: (a) Total lipids per dry wt. were 6.4, 7.1–7.2, 7.2–7.3, and 7.6% aften cultivation at 4°, 16°, 22°, and 26°, resp.; (b) Phospholipid: 8.5, 8.3–11.0, 8.7–10.4, and 9.0% at 4°, 16°, 22°, and 26°, resp.; I_2-number of phospholipid: 105.5, 80, and 53, at 4°, 16°, and 26°, resp.; inositol and its phosphate compd. were isolated from cephaline fraction. (c) Unsaponified substance was approx. equally formed in the culture at 4°–26°; 1/3–1/2 of this fraction was sterol; ergosterol and its ester were also isolated. (d) Neutral fats were glyceride and formed in parallel with lipids, and (e) Fatty acid fraction (exclusive of phospholipid): C_{18}-fatty acids were found, and the more unsatd. acids were formed by cultivating at lower temp.

37. On the violet-fluorescence substance formed by the oxidation of N^1-methylnicotinamide with alkaline ferricyanide. Kiyoshi Ueda, p. 196–198. By the oxidation of N^1-methyl nicotinamide (3-carbonyl-1-methylpyridinium) with alkaline ferricyanide, 1-methyl-3-carboxylamide-2-pyridone (I) (m. p., 217–128°) with strong violet-fluorescence as well as 1-methyl-3-carboxylamide-6-pyridone (m. p., 239–240°) without fluorescence were isolated. By boiling I with Na in MeOH for 75 hrs., the cryst. of 1-methyl-3-carboxy-2-pyridone (m. p., 183–184°) with strong violet fluorescence was obtained.

38. On the affinity of β-galactosidase. Taku Kobayashi, p. 198–202. The activity of the dialyzed β-galactosisidase of 0.5% emulsin (I), 0.3% Takadiastase (II), or water exts. of autolyzed pig pancreas (III) has been estd. with *p*-nitrophenol-β-galactoside at pH 4.0–6.0. The values of pK_m are independent of pH and the following values are obtained: $pK_{m\cdot I}$, 1.8; $pK_{m\ II}$, 3.88; $pK_{m\cdot III}$, 3.58. From the inhibition of I and II by β-glactoside (IV), pK_m of I and II for IV can be calcd. to be 1.3 and 2.6, resp., which are in good agreements with those directly obtained.

39. Studies on aneurinase. VIII. Isolation of the decomposition procucts of thamine by shell-fish aneurinase. Akiji Fujita & Eiichi Hasegawa, p. 202–205; *cf.* **18**, 62, 325, 339 (1944–46); **22**, 45, 89 (1950). As the decompoistion products of thamine (I) by shell-fish aneurinase (II) prepd. from *Corbicula sandai*, 2-methyl-4-amino-5-hydroxymethyl pyrimidine (m. p., 193.0–194.4°) and 4-methyl-5-β-hydroxyethylthiazole (m. p., 142.5-146.5°) were isolated and identified. The proposed theory that II desaminates the pyrimidine group of I is proved to be no more sustainable.

40. Ibid. X. On the physiological action of thiamine disulfide and thiothiamine. Kaoru Kaminishi. p. 205–207. Shell-fish aneurinase can almost equally decompose thiamine (I), thiamine disulfide (II), and the reduced form of II on addg. cysteine-HCl; but scarecely thiothiamine (III). The conversion of II or III into cocarboxylase by thianine-phosphorylase prepd. from "thiaminase bacilli" is about 15% of that of I. The authors have suggested that II and III may be considered to be neither active nor actual oxide form of the vitamin.

41. Ibid. XI. Fish aneurinase.

Akiji Fujita, Shunichi Kozuka, Kenko Yamazaki, & Eiichi Hasegawa, p. 207–211. The following properties of fish aneurinase have been observed with water exts. of fish organs (1:40) at pH 4.5: opt. pH, 6.0; otp. temp., 44°; rather thermostable (inactivated at 90° for 10 min. or at 80° for 25 min.); O_2 is not necessarily required for the action of I. The activity of I is remarkably accelerated by cysteine and slightly by KCN, but not by methionine: strongly inhibited by monoiodoacetate or H_2O_2. The distribution of I: generally rich in kindey, spleen, and gills; found in liver or eyeballs of some species; but poor in muscle.

42. On the synthesis of 2-keto-6-cholanic acid. Genshu Wakabayashi, p. 211–213. The reaction of hyodesoxycholic acid-methyl ester (I) with Al-phenolate yields 3-keto-6-hydroxycholanic acid-methyl ester (II) (f. p., 121–123°), which is further hydrolyzed with 0.5 *N* methanolic NaOH to 3-keto-6-hydroxycholanic acid (III) (f. p., 198–199°). By heating II in acetic anhydride, acetate compd. of II is formed; semicarbazone of II is hardly synthesized. II can be reduced to I by gassing with H_2 in the presence of Pt-oxide. 6-Hydroxycholanic acid (f. p., 227–228°) is also synthisized from II by heating with hydrazinehydrate and NaOH. The hydrolysis of the distillate obtained from II at 3 mm. Hg at 180–250° for 30 min, and at 280–305° for 1 hr. successively, yields 3-keto-cholenic acid (f. p., 184–186°), which is further reduced by H_2 and Pt-oxide to a cryst. substance (f. p., 195–196°; yellow Lieberman reaction), being most likely to be epi-allo-cholic acid (f. p., 218°) or allo-lithocholic acid (f. p., 208–210°)

43. The oxidation of desoxycholic acid by Oppenauer's method. Genshu Wakabayashi, p. 213–214. Desoxycholic acid-methyl ester (I) is oxidized to 3-keto-12-hydroxy-cholanic acid-methyl ester (II) (f. p., 144–145°) by heating I with Al-phenolate-benzol soln. By hydrolyzing II with 0.5 *N* methanolic KOH, 3-keto-12-hydroxy cholanic acid (III) (f. p., 121–122°°) is yielded. When III is dissolved in glacial AcOH. and heated with anhydrous Na-acetate, 3-keto-12-acetoxycholanic acid (f. p., 69–70°) is obtained.

44. Studies on the interreaction between hemin derivatives and O_2 or H_2O_2. I. On the process of verdohemochrome formation. Goro Kikuchi, p. 214–224. A soln. of 0.088 mg./ml. pyridinehematin (I) was prepd. by dissolving cryst. beef blood hemin in 20% pyridine. Into a cuvette 0.3 ml. of L-ascorbic aicd soln. (II) was transferred, and after gassing with N_2 for the removal of O_2, the content was covered with liquid paraffin, through which 3.0 ml. of I was added drop by drop from a burette. By this addn. I was converted into pyridine-hemochrome (III). During the aeration of this mixt. at a rate of 30 ml./min., the adsorption bands have been photometrically. detd.: III has 3 bands of $\varepsilon_{557} > \varepsilon_{535} > \varepsilon_{480}$, intensities of which are lowered by aeration, and a new band at 630 mμ appears at 2nd. min., turning to a band of verdohemochrome (IV) at 656 mμ at 20th min.; another new band is also found at 14–20th min.; the addn. of H_2O_2 (1 mg./ml.) to a mixt. of II and III forms a band at 630 mμ without aeration at an accelerated rate, which can be converted to a band of IV only by aeration; a band

at 630 mμ (V) is not reduced by an excess amts. of II, but completely reduced by $Na_2S_2O_4$, forming 2 bands of $\varepsilon_{525} > \varepsilon_{557}$; IV can not be reduced by $Na_2S_2O_4$. The increase of II upto 0.8 mg./ml. accelerates the initial rate of V formation as well as the max. value of V; IV formation is readily highest. by II in a concn. of 0.4 mg./ml.; for the complete conversion of III to IV, 20 moles. of II is required per mole. of III. Successive addns. of II accelerate the formation of V as well as that of IV. From these exptl. results the mechanism of IV formation has been explained as follows: IV formation consists of 2 step reaction; V will be an intermediary one, the formation of which needs either II or H_2O_2; further conversion to IV takes place directly by O_2, but neither by II nor by H_2O_2.

45. Studies on the fate of anthranilic acid in animal body. I. The isolation of 5-hydroxyanthranilic acid. Yoichi Shirai, p. 223–226. To rabbits 2 g. of anthranilic acid were injected and 24 hrs'. urine was collected for the isolation of a substance with purple red color reaction on addg. dil. NH_4OH: to the urine add 1/10 vol. of conc. Pb acetate, and filter; after removing pB from the filtrate by H_2 gassing, concentrate the filtrate to about 100 ml. *in vacuo* under H_2, and filter off the formed cryst. of anthranilic acid; ext. the filtrate with ether for 40 hrs. and evap.; with the evapd. residues repeat the distillations at 5 mm. Hg under H_2 gassing twice after the addn. of water and twice after the addn. of absol. alcohol, successively (if water or alcoholinsol. material separates out, remove it each time before distillation); ext. the final residue several times with warm ether, and evap. at room emp.; to the formed cryst. add glacial with a small amt. of water, and ether, AcOH, and wash successively, and evap. *in vacuo*. Thus obtained residue was identified as 5-hydroxyanthranilic acid with the following properties: m. p., 231° (decomps.); sol. in dil. HCl and dil. NaOH; insol. in water, cold alcohol, cold ether and glacial AcOH; analysis of N: found, 8.69 %, and theoretical, 9.15%.

46. On the antigenity of lipoprotein. Noriaki Yoshida, p. 227–230. Lipoprotein was isolated from mammalian livers by extn. with saline soln., pptn. with $(NH_4)_2SO_4$ at 0.5 satn., dialysis, and repeated washings of the formed ppts. with acetone. Analyses: N, 11.0; ether-sol. P, 0.85; phospholipid, 21.0 mg.% (beef liver lipoprotein (I)); N, 13.7; phospholipid, 36.6 mg.% (human liver lipoprotein (II)); N, 4.6 mg.% (rabbit liver lipoprotein (III)). I, II, III, and human liver saline exts. (IV) showed the antigenic activity for rabbits, by precipitin test as well as by cephaline-cholesterol-flocculatton test, somewhat organspecificity being observed. With the progress of necrosis caused by binding a part of rabbit liver *in situ*, both tests became positive.

47. A study on cephaline-cholesterol fiocculation test. Noriaki Yoshida, p. 230–234. The antigenic substances for cephaline-cholesterol flocculation (C.C.F.)-test have been prepd. from rabbit and human livers, beef brain and commercial soybean lecithin, and fractionated into lecithin (I) and cephaline (II). With the sera obtained from rab-

bits administered with $CHCl_3$ or yellow P, C.C.F.-test using a mixt. of cholesterol (III) and either I or II from different origins. (a) I and II from rabbit liver: only ether soln. of II is found to be available, and specific to liver lesions; (b) I and II from soybean: less available; (c) II from beef brain is enough utilizable; (d) human liver: II is available and specific to liver lesions; but I, sphingosin and II are not available.

48. On the quantitative determination of free tryptophan. Tsutomu Sekine, p. 234–235, Cf., 17, 115 (1943): *J. Biochem.*, **33**, 17 (1941). The contents of tryptophan (I) in biological fluids has been detd. as follows: To 2.0 ml. of deproteinized filtrates, with 16% CCl_3COOH, add 4 ml. of $CHCl_3$ and 1 ml. of glacial AcOH, heat in a water bath at 50° for 10 min.; after addg. 2 drops of 0.01% methyl orange, add Br-AcOH mixt. (25 ml. of Br_2 in 5 ml. of 33% AcOH) drop by drop until red color in water layer fades away; repeat this bromination by addg. dild. Br-AcOH mixt. with 33% AcOH (1:4) in the presence of 1 drop of methyl orange; transferred $CHCl_3$ layer in a calibrated tube, dilute with glacial AcOH-$CHCl_3$ mixt. (1:10) to the 10 ml.-mark, and est. the red color intensity by Pulfrich's photometer (S_{53}) or by Duboscq's colorimeter. The standard soln. of I can be replaced by a mixt. of $M/10$ $CoCl_2$ and $M/10$ $CuSO_4$.

49. Studies on the interaction between hemin derivatives and H_2O_2 or O_2. II. On the influence of cupric ion upon the process of verdiohemochrome formation. Goro Kikuchi, p. 235–240; *cf.*, **22**, 214 (1950). The addn. of $CuSO_4$ to a mixt. of 3.0 ml. of pyridine hematin (I) (0.088 mg./ml.) and 0.2 ml. of L-ascorbic acid (26.4 mg./ml.) retards the reaction of forming an intermediate substance with a max. adsorption band at 630 mμ (II) from I as well as the successive reaction converting II into verdohemochrome, and the retarding influence is increased with the concn. of Cu^{++}. In this reaction series Cu^{++} seems to form the by-products.

ABSTRACTS

from

The Journal of Japanese Biochemical Society (Seikagaku).
Volume 23 (1951)

1. Biochemical studies on dietary fats. I. On the utilization of whale- and fish-oils, and their influence upon the absorption of carbohydrate and protein. Yoshihiro Matsumura. p. 26–29. Six healthy men (22–34 years) were subjected to the supplementary administration of daily 40 g. of whale- or fish-oils in addn. to the basal diet contg. carbohydrate, 490.8 g.; fat 40 g.; protein, 90.2 g.; ashes, 23.0 g.; fresh vegetables; 2855 k. cal. During the first 2 days (control period) and successive 3 days (fat-diet period) urinary N, fecal excretion of N, fat, and hydrolyzable sugar have been estd. Fat is found to be excreted usually 3 g. a day, being independent of the kinds of administered oil. The absorption of fats is calcd. to be more than 90% with 4 whale oils, 2 fish oils (sardine, and shark), and rice-bran oil. Fecal excretion of carbohydrate and protein (N) is less influenced even by supplying a large amt. of fat.

2. Colorimetric determination of blood sugar. Hiroshi Ito. p. 29–32. Mix 0.1 ml. of blood with 10 ml. of water, add 0.1–0.15 g. of borax-Zn acetate (7:4), centrifuge; to 2.0 ml. of the deproteinized supernatant add 1 ml. of 0.1 *M* Na_2CO_3-NaOH mixt. and 1.0 ml. of 0.25% K-ferricyanide; heat in a boiling water bath for 15 min., cool; add 1 ml. of 0.25% iron alum dissolved in 0.5 *M* HCl; 30–40 min. later in dark, add 2 ml. of 10% KNO_3, let stand for 10–15 min., centrifuge for 10 min.; dissolve the ppt. in 1 ml. of 0.07% oxalic acid, dilute to 10.0 ml. with water; measure the formed berlin-blue color by Pulfrich's photometer with S_{61}. Calcuation : blood sugar $= (\varepsilon_{\text{sample}} - \varepsilon_{\text{blank}}) / 3.85$ g. %. Recovery : 100∼101.0 %; glutathione and creatine do not interfer; 20–70 mg.% creatinine causes 2–6 mg.% of color intensity as sugar.

3. The molecular weight of nucleic acids. Masakazu Tsuji. p. 32–35. With purified yeast nucleic acid (Merck) (I), β-nucleic acids of calf spleen (II) and of cod sperm (III) prepd. by using Pancreatin, the diffusion const. has been detd. at 25° by Lamm-Polsen's method : I, $42.5 \sim 42.8 \times 10^{-7}$; II, 45.8×10^{-7}; III, 44.5×10^{-7} cm²./sec. α-Nucleic acids of calf spleen and cod sperm give an asymmetric diffusion pattern. Applying a formula of colloide molecule, mol. wt. of I is calcld. to be 750, while the theoretical value is given to be 1286 as tetranucleotide. The discrepancy is considered to be due to more highly spheric shape of I, suggesting the ring structure of its tetranucleotide.

4. Studies on nucleic acid by electric titration. Ibid. p. 36–37. Yeast nucleic acid, α- and β-nuclic acids prepd. from calf spleen or cod sperm give pK= 8.2, for which 4 mols. of NaOH are required per mol. of nuclic acid, and the last 1 equiv. of NaOH is titrated from

pH 5.5 to 8.2, indicating the 4th primary phosphoric acid group in the tetranucleotide structure.

5. Studies on the interaction between hemin derivatievs and H_2O_2. or O_2. III. The decomposition of pyridine-hematin by H_2O_2. Goro Kikuchi. p. 38–44; *cf.*, **22**, 214, 235 (1950). By the addn. of H_2O_2 to pyridine-hematin (I) (20% pyridine) bands of I at 540 and 570 mμ are reduced, which are replaced by a vast adsorption band at 600 mμ, but there are not formed the bands of verdohemochrome at 630 and 656 mμ. On reducing with $Na_2S_2O_4$ bands at 557 and 525 mμ appear. The reaction between I and H_2O_2 is accelerated proportionally to H_2O_2 concn., and ε_{525} and ε_{575} are diminished with the increase of H_2O_2 as well as with the timed between H_2O_2- and $Na_2S_2O_4$-addns. The efficiency of H_2O_2 to decomp. I is rather decreased with higher concn. of H_2O_2, presumably due to the catalatic decompn. of H_2O_2 by I. In 60% pyridine and under anærobic condition, the band at 600 mμ appears immediately, which turns to bands at 536 and 532 mμ in 30 min., and finally reddish substance with bands at 557 and 525 mμ is formed, where ε_{557} is greater than ε_{525} on the contrary to those produced in 20% pyridine. In 100% pyridine bands at 587 and 566 mμ are observed on addg. H_2O_2 within 30 min. which are conjugated to a vast band at 500~590 mμ. The new adsorption bands formed in higher concn. of pyridine are considered to be due to the decompn.-product of I by H_2O_2.

6. Ibid. IV. On the reaction of pyridine hemin - ascorbic acid - H_2O_2 system. Goro Kikuchi. p. 44–51. The following reaction has been photometrically examined : pyridine-hemin (I) (20% pyridine) (557 and 525 mμ) + ascorbic acid (II) + H_2O_2 → intermediary substance (630 mμ) (III) + $Na_2S_2O_2$ → pyridine-hemochrome (IV). (557 and 525 mμ). On reduction ε_{557} and ε_{525} are decreased, but ε_{557} / ε_{525} is rather increased with the increase of ε_{630}. The formation of III is increased with the concn. of H_2O_2 or II : 50% formation by 1.5×10^{-4} *M* H_2O_2; opt. concn. of II at $0.5 \sim 1.0 \times 10^{-2}$ *M*; opt. pH at 9.3. The values of ε_{557} / ε_{525} of IV on 100% formation of III, and of pure I are 0.75, and 1.90, resp., from which $\varepsilon_{\text{mol. } 630}$ of III is calcd. to be 0.82×10^{-2}. The ratio, II/H_2O_2 has its max. value at rather lower concn. of H_2O_2 ($10^{-4.8}$ *M*), suggesting catalatic or peroxidatic decompn. of H_2O_2 by I and more strongly by II. By peroxidatic reaction of I with H_2O_2, III is formed, and subsequently III reacts with H_2O_2 catalatically or peroxidatically. The role of II is considered to give favourite redox potential to yeild appropriate amts. of Fe^{+++} and Fe^{++}, resp. for the formation of III.

7. Metabolism of anthranilic acid in animal body. II. A new color reaction of 5-hydroxyanthranilic acid. Yoichi Shirai and Shushi Uno. p. 51–56; *cf.*, **22**, 223 (1950). On addg. 0.5 ml. of 0.1 *N* KI to 5-hydroxyanthranilic acid (I) soln. previously adjusted to pH 1 with 0.3 *N* HCl, violet-red color appears immediately; red color by high concn. of I (1 mg.%). The colored substance is not extd. with ether. This color reaction can be differentiated from other interfering substances. (a) Adrenalin:

far slower colorization; (b) The color formed by the following substances can be extd. with ether : 3-hydroxyanthranilic acid, *m*-aminosalicylic acid, cysteine, ascorbic acid, tryptophan, *o*-and *p*-aminophenol, anthranilic acid, catechol, hydroquinone, resorcinol, pyrogallol, and orcinol. (c) Not colored substances : arginine, tyrosine, histidine; thiamine, riboflavnie, nicotinic acid, vitamin B_2; phenol, salicylic, hippuric, and henzoic acids, aniline, *o*- and *p*-aminophenylsulfonamide. (d) Not colors with normal human or rabbit urine constituents. The color intensity becomes max. in 9 min, and lasts const. for 60 min. By use of a yellow filter (Riken No. 2) Lambert-Beer's law is satisfied. With rabbit urine the best recovery can be obtained under the following procedures: (a) Removal of urine pigments and colloidal substances by phosphotungstate; (b) Removal of anthranilic acid with an equal vol. of ether; (c) Color development at pH 2.5 by addg. Na-acetate.

8. Ibid. III. On the 5-hydroxyanthranilic acid-forming position in rabbit. Yoichi Shirai, Takao Sasaoka, Shushi Uno, Yushi Higashi, Sadasuke Takeda, and Tsunehito Arai. p. 56–58. After subcut. injection of 2.0 g. of anthranilic acid (I) to rabbit, every hour's urinary excretion of 5-hydroxyanthranilic acid (II) has been detd. as follows: 3.65, 10.04, 11.68, 18.55, 13.71, 12.03, 9.02, 9.64, 4.07 and 3.25 mg., resp. With the slice of rabbit organs killed at 3rd. hr., the argylphile granules are examd.: epithelial cells of kidney tubules (###～##), parenchymal cells of liver (III) (##), striated muscle cells (±). After incubating each slice with 1% I in Ringer-Tyrode soln. mixt. at 38° for 3.5 hrs., only III shows remarkable argylphile granules. After carrying out the procedure of II isolation (**22**, 223 (1950)) with the incubated slices, only III gives pos. KIO_3 or indophenol test for II.

9. Studies on radish amylase. III. On the influence of ascorbic acid upon radish amylase. Taiji Nishida. p. 59–63. Radish amylase (I) was prepd. as follows: from press juice of radish roots ppt. I with tannin (final concn., 1%), dissolve in water, filter; ppt. with 1.5 vols. of acetone, wash with acetone, and ether, succesively, and dry *in vacuo*. The activity of I or saliva (1: 10) for 1% sol. starch has been detd. iodometrically by Willstatter and Schudel's method at pH 5.4 at 37°. Both I and II are inhibited by L-ascorbic acid (III), but neither by dehydroascorbic acid nor by cysteine (IV). Crude I in the original juice is not inhibited by III. The lost activity of I by III can be almost completely restored by the addn. of IV.

10. The determination of reactive hydrogen atom in guanosine and yeast nucleic acids by use of heavy water. Masao Uchida. p. 63–67; *cf.*, **18**, 383, 387, 391, 396, 406 (1944–47); **23**, 32, 36 (1951). With purified guanosine (m.p., 237°) (I) and Na salt of yeast nucleic acid (II) (analyzed to be $C_{38}H_{43}O_{28}Na_4N_{15}P_4$) the increments of dry wts. of I or II at 110° at 5 mm. Hg for 2～12 hrs. after dissolving in 1 ml. of 99.5% heavy water have been estd. at the intervals of 2 hrs. The replaced H atoms by D per mol. of I, and II are found to be 6, and 12, resp. The most probable positions of H atoms of I to be replaced

by 6 atoms of D are H atoms of -OH, and $-NH_2$ groups in pyrimidine and 3 -OH groups in ribose (III). If this is the case with II that has been proved to be a tetranucleotide, 12 atoms of the replaced D must be distributed as follows: each 2 H atoms of $-NH_2$ groups in adenine (IV), cytosine (V), and guanine (VI) (total, 6); each 1 H atom of -OH groups in VI and uracil (VII) (total, 2); each 1 H atom of -OH groups at C_2 of III in IV, V, VI, VII (total, 4); no H atom is more replaceable by D in 4 mols. of phosphoric acid (VIII) of II, since each H atom of the 3rd. -OH group of VIII is readily satrd. by Na atom by neutralization. It may be suggested that each 2 remaining H atoms of -OH groups in VIII do not exist more, but may be linked by 2 ester combinations between P atom and C_2 or C_2 of III to form the ring structure of II.

11. The action of periodic acid on yeast nucleic acid. Masao Uchida. p. 67–72. Adenosine (I), yeast adenylic acid (II), purified yeast nucleic acid (III), and Na salt of III are reacted with *M*/80 KIO_4 in 88% ortho-phosphoric acid for 5 hrs. under cooling, and the remaining KIO_4 is detd. by iodometric titration. Only I undergoes 1 equiv. of oxidation by KIO_4. From the reactivity of KIO_4 causing "glycol cleavage reaction" in the compd. possessing adjacent 2-OH groups., it has been concluded as follows: (a) the ribose molecule (IV) in I may form a furan ring between C_2 and C_2, and may have each 1 free -OH group at C_2 and C_2; (b) II in IV may have no adjacent -OH group, but there may be 2 linkages between a free -OH at C_2 or C_2 and a free -OH group of one of phosphoric acid molecules; (c) III in IV may be in combination with 2 mols. of phosphoric acid at C_2 and C_2, resp.

12. The distribution of riboflavin in frog eye-balls. II. Kunio Yagi. p. 72–74; *cf.*, **22**, 143 (1950). Finely cut tissues are extd. with warm water at 80° for 3—5 min., homogenized, and warmed again at 80° for 15 min. After removal of protein by satg. $(NH_4)_2SO_4$, riboflavin (I) is extd. with ether, and successively with water, and participated into FMN, FAD, and free I by use of paper chromatography (**22**, 249 (1950); *J. Biochem.*, **38**, 161 (1951)) or of paper chromatopile. (*Igaku to Seibutugaku*), **17**, 2 (1950)). With eye-bals of *Rona catesbiana*, *Rona nigromaculata*, and *Bufo vulgaris*, the following distributions have been obtained: (a) chorioidea contains much I, almost in free type; 4 blue or violet fluorescent substances are detected, likely to be pterin derivs.; (b) Retina contains only FMN.

13. On the decomposition of dietary fats by pancreas lipase. Mitsuyuki Shimizu. p. 103–105. Glycerol-water (70%) ext. of acetone-dried powder of pig pancreas was dild. 5 times with dist. water, and centrifuged. The activity of the supernatant fluid (I) was examd. in the following mixt.: I, 2 ml.; *M*/15 phosphate buffer at pH 8.05, 5 ml.; 0.1% egg albumin, 3 ml.; 0.01 *N* CaCl, 3_2 ml.; Na cholate, 2 ml.; fat specimen, 1 g. (suspended). After incubation at 37° for 3 hrs. by occasional shakings, each 10 ml. of EtOH and ether were added to the reaction mixt., and the acidity increments were titrated with 0.1 *N* NaOH. The decompn. of several dietary fats by

I were as follows: rice-barn oil>ricinus oil>margarine (whale oil)>sardine oil> shark oil>whale oils.; in case of hydrogenated sardine oil, the grade of decompn. was increased with I_2-values.

14. The activities of organic inner-complex with metal as the enzyme-model. Koichi Anan. p. 105–107. With 5 salicylaldehydeethylenediimine-complexes with metal([Me]), peroxidase, catalase, and ascorbidc acid oxidase activities have been examd. by estg. purpurogallin number (P.N.), O_2 evolution from H_2O_2, and O_2 uptake resp., in pyridine-water mixt. at pH 6.8. (a) P.N.: $[Cu^{++}] \gg [Mg^{++}]$, $[Mn^{++}] \gg [Fe^{+++}]Cl$, $[Fe^{+++}]$ acetate (almost nil); markedly by $[Cu^{++}]$+pyridine; moderately by Fe^{+++} (b) Catalase activity $[Cu^{++}] \gg [Mg^{++}]$, $[Mn^{++}] \gg [Fe^{+++}]Cl$, $[Fe^{+++}]$ acetate; considerably by Cu^{++}+pyridine. (c) Ascorbic acid oxidase activity: $[Cu^{++}] > [Fe^{+++}]Cl$, $[Fe^{+++}]$ acetate $\gg [Mg^{++}]$, $[Mn^{++}]$; $Cu^{++} \gg Mg^{++}$.

15. The concentration of *p*-aminosalicylic acid in blood and organs, and its excretion in urine. Tomoya Sasaki. p. 107–116. After the oral administration of 2.0 g. of *p*-aminosalycylic acid (PAS) to patients (tuberculosis), the blood PAS level becomes max. (2.2 mg.%) at 1st hr., and is reduced to 0.56 mg% at 4th hr. Successive administration of each 2.0 g. of PAS 6 times at the intervals of 4 hrs. results higher and delayed decrease of blood PAS level. 3 hrs. after intramusc. or intraven. injection of 1.0 g. of PAS, blood PAS level is readily below 0.1 mg.%. In 24 hrs'. urine after receiving 2.0 g. of PAS orally, the recoveries are 42.3% as free PAS and 86.8% as free PAS+acetylated PAS. After intraven. injection, the urinary excretion and blood level of PAS are decreased in geometrical progression, presumably due to PAS clearance by kidney as well as to irreversible acetylation of PAS in body. The distribution of PAS after interperiton. injection to guinea pig (10 mg. of PAS per 100 g. body wt. per day) is detd. as follows: kidney>liver>spleen>blood> lung>muscle$\gg$brain.

16. On the inhibitory effect of various drugs on the respiration of *Trichomonas foetus*. Harutada Ninomiya & Suzuoki-Iiro. p. 116–120. The cells of *T. foetus* cultivated in the medium contg. 1% peptone, 1% glucose, 10% bovine serum, and 0.5% NaCl at 37° for 48 hrs. were collected by centrifugation and suspended in the original culture medium. With 1.6$\sim$1.8 ml. of this suspension (I), contg. about 10^7 counts of cells or 15 mg. dry wt., the inhibitory effect of drugs on O_2 uptake was measured at pH 7.0 at 30° as follows: strongly inhibited by carbol, gentiana violet, merzonin, monoiodoacetate, octylalcohol, $HgCl_2$, NaF and tricholysin; but not by KCN, NaN_2, NH_2OH, malonate, quinine, atabrin, and plasmochin, With each inhibitor, O_2 uptake was still observed, even after the mobility of *T. foetus* had stopped.

17. On the influence of low protein diet upon the protein contents in liver and serum. Hidematsu Hirai, Kazuo Shimao, & Hisako Kosugi. p. 120–122. Rats have been fed either with control diet (I) (protein, 16%) or with low protein diet (II) (protein, 3%) for 2$\sim$14 days. (a) 0, 3, 7, and 14%

body wt. decreases: after feeding on II for 2, 4, 7, and 14 days, resp.; daily 3% increase by feeding on I. (b) The decreases in protein contents of organs: spleen>liver>serum>kidney; in liver and spleen the rate of the decrease is nearly parallel with the days fed on II. (c) Organ wt. decreases: serum> liver>spleen; insignificantly in kidney. (d) Water contents of liver is increased together with non-protein solid components (III), most of the latter being perhaps due to the increase in fat component. (e) Serum albumin is decreased in approx. proportion to that in liver protein; but relative increases in α- and β-globulins. Fasting (receiving only water) causes no significant change in spleen, liver, and kidney protein contents, but great decreases in water content and in III of liver are observed. Protein is found to be mutually supplied between liver and spleen, but not to and from kidney.

18. The decomposition of horse serum by Taka-enzymes, and the specificity of its end-products. Hidematsu Hirai. p. 122–125. To 2.5 times dild. horse serum, Taka-diastase powder (Commercial) was added to a concn. of 0.5%. During the incubation of this mixt. at 37° at pH 5.0, an aliquot has been pipetted out to detn. amino-N and the reducing powder. Amino-N is increased to max. value on 15–20th day after an initial lag period for 0–5 days. The max. reducing power is observed on about 20th day. The control serum (I) incubated with heat-inactivated Taka-diastase, and the treated serum (II) after 20 days' incubation are fractionated with Na_2SO_4: (a) N.P.N.—I, 5; II, 68 mg.%. (b) I contains about 50% less albumin, and relatively high concn. of globulin; but in II only pseudoglobulin fraction is found. From the rabbit immunized with I and II, separately, antiprecipitin (I′, and II′, resp.) were obtained and showed the precipitin test as follows: I+I′ (##); II+II′ (##); I+II′ (+); II+I′ (±). With the immunized rabbit the results of Arthus's phenomenon and anaphylaxy were nearly analogous to those of precipitin test.

19. Chemical components of the bile juice of the bacillus carriers. II. On the problem of the existence of taurocholic acid in human bile juice, and the isolation of glycoanthropodesoxycholic acid. Kazumi Yamasaki. p. 125–129. By treating human bile juice with solvents, followed by pptn. with $FeCl_3$, there were found 2 conjugated cholic acids: glycocholic acid, 0.39%; glycoanthropocholic acid, 0.41%; but no appreciable amt. of taurocholic acid was obtained; no free bile acid was found on extg. with ether in strong mineral acid soln. The bile juice of the carriers showed the same chemical components.

20. On the action mechanssm of human blood cholinesterase. I. The difference of pS-activity curves between human serum and erythrocyte cholinesterases. Ryoiti Shukuya. p. 129–133. Serum is separated from 2 ml. of blood and dild. 2 times with Ringer soln. Serum (serum cholinesterase (I)). Erythrocytes are thrice washed with Ringer soln., and dild. to 10 ml. with Ringer soln., (erythrocyte cholinesterase (II)). Enzyme activity is detd. manome-

trically in bicarbonate buffer for acetylcholine (III). Michaelis const.: I, 1.2×10^{-3}; II, 3.7×10^{-4} mol./lit. Excess III inhibits the activity of I, by forming the inactive complex (ESS) in $ES+S\rightleftarrows ESS$ (E, I; S, III); the diss. const. of ESS is calcld. to be 1.2×10^{-2} mol./lit.

21. Ibid. II. Inhibition mechanism of human blood cholinesterase by eserine. Ibid. p. 133–136. The inhibitory action of eserine (IV) is observed on both I and II by the same mechanism to form the inactive complex (EG) in $E+G\rightleftarrows EG$ (G, IV; E, I or II). Diss. const. of EG: I, 0.9×10^{-8}; II, 0.88×10^{-8} mol./lit.

22. Quantitative studies of the choleglobin forming-reaction. Goro Kikuchi. p. 137–141; *cf.*, **22**, 214, 235 (1950); **23**, 38, 44 (1951) The reaction: hemoglobin (I) + ascorbic acid + $O_2\rightarrow$ choleglobin (II) (670 and 630 mμ for ferric and ferrous II, resp.)$\rightarrow$cholehemochrome (green ppt.) has been quantitatively investigated. On addg. $Na_2S_2O_4$, and NaOH, successively, I and II or III are reduced to alkalihemochrome (IV) (557, 527 mμ) and alkalicholehemochrome (V) 618 mμ), resp. From the detd. values: ε_{557} of IV=3.5; ε_{618} of V=1.6; $\varepsilon_{\text{mol. }618}$ of choleheme group in III or IV is calcd. to be 1.36×10^4.

23. Studis on the metabolism of cholic acid. I. Nobuaki Takemoto. p. 142–146. (a) Daily 60 ml. of 1% Na cholate (I) were administered orally to 2 rabbits through a catheter into stomach for 42 days. From the collected urine (10068 ml.) 20 mg. of desoxycholic acid and 122.3 mg. of chenodesocycholic acid (II) were obtained. From 272 g. of feces (dry wt.) 109.3 mg. of II and an undetermined bile acid (m.p., 192°; Libermann test (—); nitroprusside test (—); Hammarsten test (+) were isolated. Glycodesoxycholic acid was identified in bile juice. In liver a small amt. of I was found. (b) Dogs were subjected to daily 166 ml. of Na dehydrocholate (III) for 15 days, by a catheter into stomach. From 5911 m. of urine 30 mg. of III, and 200 mg. of α-3-hydroxy-7, 12-diketocholanic acid were isolated. From 82 g. of feces 200 mg. of 3,7-dihydroxy-12-ketocholanic acid was obtained.

24. A new color reaction of carbonyl compounds. Yasushi Koide. p.146–151. Carbonyl compds. were found to develop yellow~reddish brown color on addg. chloral hydrate, Na_2CO_3, and pyridine, followed by heating in a boiling water-bath under liquid paraffin. This color reaction was positive with mono- and di-saccharides possessing reducing power, aliphatic or aromatic aldehyde- and ketone-compds., keto acids, ethyl- and butyl-acetate. The highest extinction coeff. was obtained by Pulfrich's photometer with S_{43} in the following reaction mixt.: 100 mg./dl. hexose, 0.5 ml.; 0.1 *N* NaOH, 10 ml.; 0.05 *M* chloral hydrate, 1 ml.; pyridine (b.p., 114.8–115.1°), 1 ml. Const. and stable color was obtained by heating for 20 min. The color intensity was increased with pH or by the addn. of nitrobenzol, but rather decreased by high concn. of urea (more than 1%); the simultaneous addn. of nitrobenzol and urea delayed the color development and minimized the change in color intensity.

25. Studies on vitamin B_2 administered to rats. Kunio Yagi. p. 151–153. (a) The normal values of flavine compds. in rat organs were detd. separately by warm water extn., partition paper-chromatography, and lumiflavin method. (*cf.*, **22**, 240 (1950); *J. Biochem.*, **38**, 161 (1951)). (i) Total flavine compds. (I): kidney liver heart. (ii) Each organ contains FAD mainly; a slight amt. of FMN and free riboflavin (II). (b) The distribution of I 30, 60, and 120 min. after subcut. injection of II (300 γ) to rat: II and FMN are increased in intestine; FAD is increased in liver and kidney. (c) 60 min. after FMN injection (300 γ as II): far less change in intestine, except for a little increase in FMN; FAD is considerably increased in liver and kidney. (d) 60 min. after FAD injection (300 γ as II): FAD and FMN are increased in intestine; FAD is remarkably increased in liver and kidney, and slightly in heart. Thus II is found to be converted to FMN in intestine, which undergoes further change to FAD in liver or kidney.

26. Studies on aneurinase. XV. On the activation of shell- or fish-aneurinase by organic base compounds. Shunichi Kozuka. p. 154–163.; *cf.*, **18**, 62, 325, 339 (1944–46); **22**, 45, 89, 202, 205 (1950). With shell-(I) or fish-aneurinase (II) prepd. by water extn. (1 : 10) at pH 4.5, the activation of *M*/200 organic base compds. has been fluorometrically detd. at pH 5.5 in the presenc of 1 γ of thiamine. The activation of I is usually more than 10 times as much as that of II. (a) Aromatic compds.: aniline; *m*-$>$*o*-$>$*p*-NH_2 dervs.; as substituted radicals, -$NH_2$$>$-$CH_2$$>$-OH$>$-COOH$>$-$NO_2$, but not by -NHR or -NRR′. (b) Heterocyclic compds.: imidazole$>$quinoline$>$pyridine; α-NH_2-β-NH_2-$>$free-pyridine; not by NO_2^- and HSO_2-pyridine; appreciably by NH_2-thiazole; slightly by 4-OH-pyrimidine, but rather inhibited by 4-amino-pyrimidine; NH_2-derivs. of purine; strongly inhibited by thiamine derivs. (d) SH-compds.: γ-aceto-γ-mercapto-propyl-alcohol or- acetate$>$L-cysteine$>$NaHS$>$glutathione$>$$Na_2S_2O_4$. (e) Activating effect of aniline is depressed by 4-NH_2-pyrimidine, but not by 4-OH-pyrimidine.